214

PROCEEDINGS

OF THE

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EXPERIMENTAL BIOLOGY AND MEDICINE

INCLUDING THE
PACIFIC COAST BRANCH, MINNESOTA BRANCH,
WESTERN NEW YORK BRANCH, AND
PEKING (CHINA) BRANCH.

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E. V. McCOLLU

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

One hundred thirty-third meeting.

New York Post-Graduate Medical School, October 17, 1923.
President Jackson in the chair.

1 (2233)

Demonstration of the Vernes flocculation test for syphilis.

By ADELAIDE B. BAYLIS, ADELE E. SHEPLAR and
WARD J. MAC NEAL.

[*From the Department of Pathology and Bacteriology, New York Post-Graduate Medical School and Hospital, New York City.*]

Fifteen to twenty cc. of blood are taken in the usual manner from a vein of the patient and collected in a test tube. The tube is then stoppered and placed in the refrigerator over night to allow the serum to separate. The serum is then decanted and centrifugalized for two minutes at high speed to free it from all traces of corpuscles; the clear serum being transferred to a fresh tube which is tightly closed with a rubber stopper and inactivated one-half hour at 55°C and allowed to cool for one-half hour at room temperature.

The antigen is prepared with the mechanical aid of the mélangeur: 6 cc. of the specially prepared alcoholic extract of horse's heart, called péréthynol, are dropped at the rate of 1 cc. per minute into a beaker containing 12 cc. of doubly distilled water, the suspension being stirred at the rate of 200 revolutions per minute. (See Fig. 1.) After the temperature of this suspension, which has been elevated by the stirring, has regained its original temperature, 21 cc. of doubly distilled water are added very slowly by hand, while the suspension is being mixed at the same rate, and the stirring continued a few minutes after all the water has been added.

It is most important that all solutions should be of the same temperature, between 20° to 25°C, before adding one to the other, and that the doubly distilled water be prepared in all-glass apparatus free from rubber or metal connections. For the test four tubes are set up for each patient and 0.8 cc. of inactivated serum placed in each tube. To two of these (the reaction tubes) 0.4 cc.

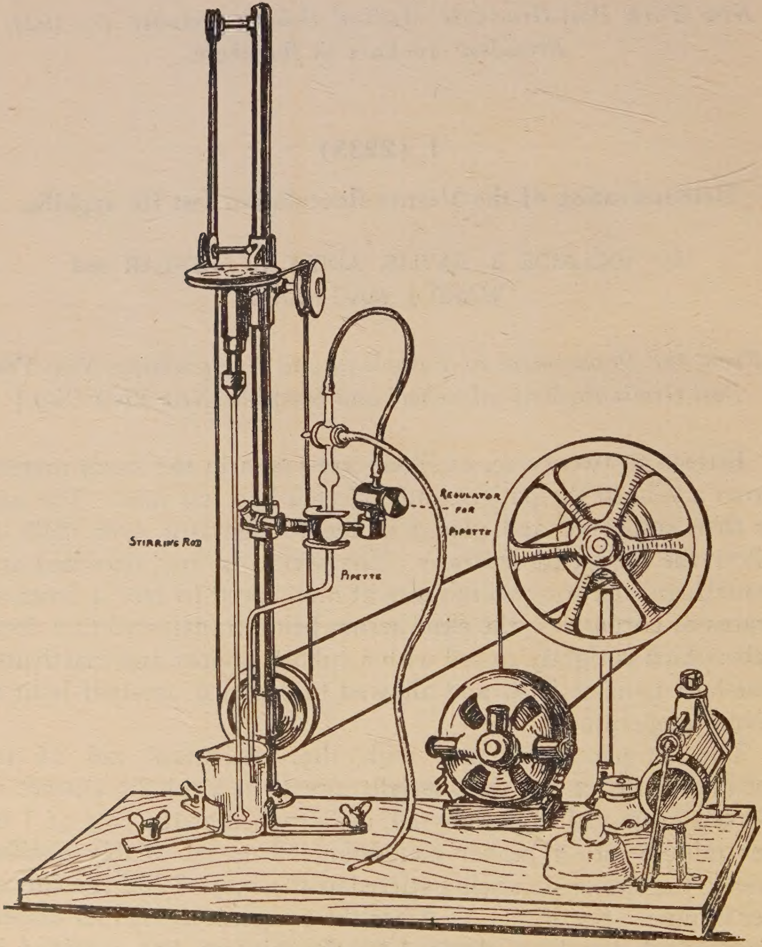

FIG. 1—Vernes Mélangeur.

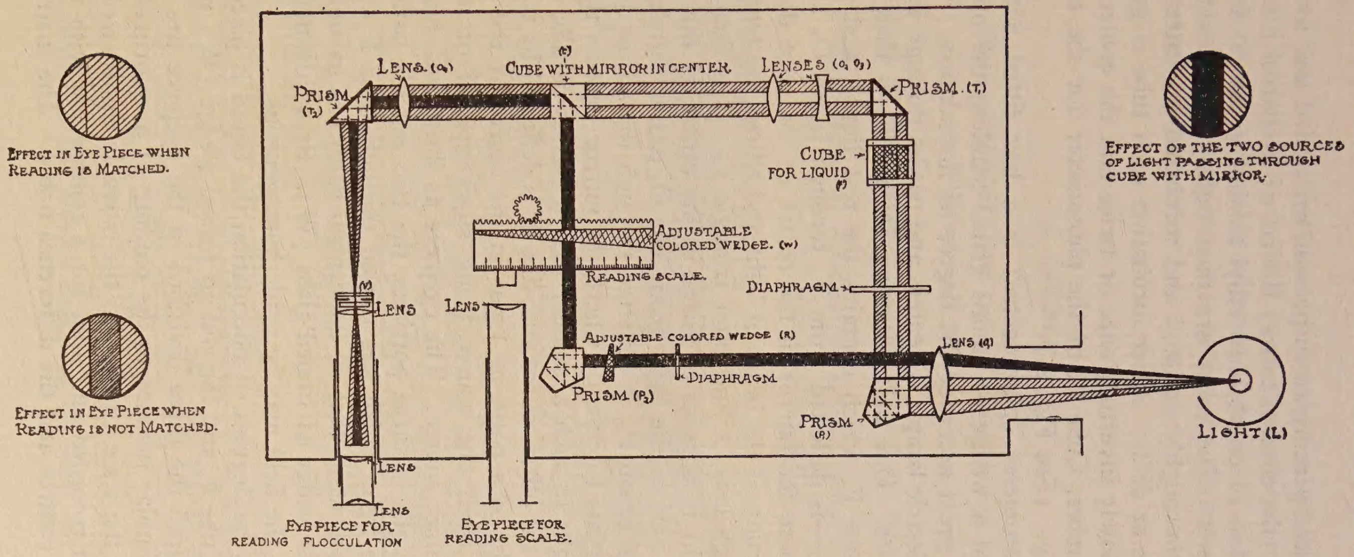
Effect in Eye Piece when Reading is Matched.
Effect in Eye Piece when Reading is not Matched.
Prism (T₂)
Lens. (O₄)
(E)
Cube with mirror in center.
Lenses (O, O₃)
Prism. (T₁)
Cube for liquid (F)
Adjustable colored wedge. (W)
Reading scale.
Diaphragm
(V)
Lens
Lens
Adjustable colored wedge (R)
Diaphragm
Prism (P₂)
Lens (Q)
Prism (P₁)
Lens
Lens
Eye piece for reading flocculation
Eye piece for reading scale.
Effect of the two sources of light passing through cube with mirror.
Light (L)

FIG. 2—Photométre Vernes, Bricq, and Yvon.

of the diluted péréthynol suspension are added and to the other two tubes (the control tubes) 0.4 cc. of a control diluted alcoholic solution (1 cc. absolute ethyl alcohol to 5.5 cc. doubly distilled water). The tubes are then tightly closed with rubber stoppers, thoroughly agitated and incubated in water bath for four hours at 25°C. After incubation each tube is gently agitated by slowly inverting once or twice and the contents placed in the chamber, Cube F, of the photometer for the estimation of turbidity. (See Fig. 2.)

The photometer (photométre) is a box fitted with lenses, prisms, and a wedge of fumed glass, enabling the operator to read with great accuracy the degree of flocculation. The light from an electric lamp (L) enters and passes through lens O_1, in parallel lines. One half, entering Prism P_1, is then deflected through cube F, which contains the test liquid, and passes to triangle T_1—is deflected from T_1 through lenses O_2 and O_3 to cube C where the mirror in the centre of the cube deflects the central portion of the beam of light and allows the remainder to pass through lens O_4 and enter triangle T_2. It is then deflected and brought to a point of focus in the centre of the eyepiece; The other half of the light from lens O_1 passes through fumed wedge R to prism P_2, is deflected through the adjustable wedge of fumed glass to cube C, where the mirror deflects the central band of light in such a manner that it passes parallel with the light from the other source and, like it, is deflected by triangle T_2 and brought to a point of focus in the centre of the eyepiece; the beam from one source being superimposed on the beam from the other source. The eyepiece as illustrated receives the beams of light in three portions, the two outside bands representing the light which has passed through cube F containing the test fluid, while that of the central band has passed through the movable wedge of fumed glass (W). By adjustment of this wedge, W, the field may be made homogeneous.

To read the degrees of flocculation the liquid is placed in the chamber, Cube F, and the sliding fumed wedge (W) moved by a ratchet until the three divisions in the eyepiece are homogeneous, in similar manner to the reading of a colorimeter. The number on the scale attached to the movable fumed wedge (W) is read and recorded and used for a comparison with the reading of the control and the difference noted. The instrument is so designed that this difference represents the amount of flocculation or precipitation in milligrams per cubic centimeter of fluid. There are several accessory mechanical features and minor details of the test, which it is not our purpose to discuss in the present communication.

2 (2234)

The effect of loss of sleep on the composition of the blood and urine.

By NORRIS W. RAKESTRAW and FLORENCE O. WHITTIER.

[*From the Department of Chemistry, Leland Stanford Jr. University, Stanford University, Calif.*]

The experiments were carried out on eight normal men, on a uniform diet, who remained awake over a period of 48 hours. Twenty-four hour urine samples were collected for two days of the wakeful period, as well as the normal day preceding. Blood samples were taken at the beginning, middle and end of the period. The whole experiment was repeated after an interval of three weeks, the men alternating as controls and subjects in the two periods.

Total nitrogen, urea, ammonia, chlorides, phosphates, uric acid and total acidity were determined in the urine by the customary methods; while in the blood, determinations were made of sugar, non-protein nitrogen, urea, phenols (both free and conjugated), uric acid, chlorides, and alkali reserve. In four cases total "acid soluble" phosphorus and lactic acid were also determined in the blood.

The results showed no really consistent alteration in any of the components studied with the exception of a possible increasing tendency of the total nitrogen and the phosphorus of the urine and an increase in the lactic acid and phosphorus of the blood. The data concerning these last two components are too fragmentary, however, to justify any positive conclusion as yet.

The experiment has shown that loss of sleep for as long as forty-eight hours does not produce any considerable alteration in the composition of human blood or urine.

The investigation is being carried further.

3 (2235)

Equilibria involving calcium, hydrogen, carbonate, bicarbonate, primary, secondary and tertiary phosphate ions.

By I. NEWTON KUGELMASS and A. T. SHOHL.

[*From the Department of Pediatrics, Yale University, New Haven, Conn.*]

The calcium salts of bicarbonate, carbonate, and primary, secondary and tertiary phosphates were studied to ascertain the conditions governing the equilibria at 38°C. Such a system may be evolved theoretically and experimentally by the addition to water of CO_2, $CaCO_3$, Na_2HPO_4. The resulting equilibria may be expressed by the equations

$$CO_2 \text{ (gas)} \rightleftarrows CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows \overset{+}{H}\ \overset{-}{HCO_3} \rightleftarrows \overset{+}{H}\ \overset{=}{CO_3}$$

$$CaCO_3 \text{ (solid)} \rightleftarrows CaCO_3 + H_2CO_3 \rightleftarrows Ca(HCO_3)_2 \rightleftarrows \overset{++}{Ca} + 2\overset{-}{HCO_3}$$

$$\text{combining}\ \left[\overset{+}{H}\right] + \left[CaCO_3\right] \rightleftarrows \overset{++}{Ca} + \overset{-}{HCO_3}$$

This resulting equilibria may be expressed according to the mass action law as

$$K_0\,[CO_2] = [H_2CO_3]$$

$$K_1\left[H_2CO_3\right] = \left[\overset{+}{H}\right]\left[\overset{-}{HCO_3}\right]$$

$$K_2\left[\overset{-}{HCO_3}\right] = \left[\overset{+}{H}\right]\left[\overset{=}{CO_3}\right]$$

$$K_3 = \left[\overset{++}{Ca}\right]\left[\overset{=}{CO_3}\right]$$

$$K_4 = \frac{K_1K_3}{K_2} = \frac{\left[\overset{++}{Ca}\right]\left[\overset{-}{HCO_3}\right]^2}{[H_2CO_3]}$$

$$\text{and } K_5 = \frac{K_4}{K_1} = \frac{K_3}{K_2} = \frac{\left[\overset{++}{Ca}\right]\left[\overset{-}{HCO_3}\right]}{\left[\overset{+}{H}\right]}$$

If we introduce Na_2HPO_4 into such a system

$$HPO_4^{=} + Ca^{++} \rightleftarrows CaHPO_4 \text{ or}$$

$$K_6 = [Ca^{++}]\,[HPO_4^{=}]$$

Multiplying K_5 by K_6 we obtain

$$K_5 \cdot K_6 = \frac{[Ca^{++}]^2\,[HCO_3^{-}]\,[HPO_4^{=}]}{[H^{+}]} = K_7$$

The complete equilibrium involving $CO_3^{=}$ and $PO_4^{\equiv}$ takes place when the solution is made more alkaline. This relationship may be derived from the equation

$$HPO_4^{=} \rightleftarrows H^{+} + PO_4^{\equiv} \text{ or}$$

$$K_8 = \frac{[H^{+}]\,[PO_4^{\equiv}]}{[HPO_4^{=}]}$$

Multiplying K_8 by the square of K_7 we obtain

$$K_8(K_7)^2 = \frac{[Ca^{++}]^4[HCO_3^{-}]^2[HPO_4^{=}]\,[PO_4^{\equiv}]}{[H^{+}]} = K_9$$

Multiplying this by the solubility product constant of $CaCO_3$, K_3, we obtain

$$K_9 \cdot K_3 = \frac{[Ca^{++}]^5\,[HCO_3^{-}]^2\,[CO_3^{=}]\,[HPO_4^{=}]\,[PO_4^{\equiv}]}{[H^{+}]} = K_{10}$$

The above equation for the complete equilibria is not applicable until better values for the third dissociation constant are available. Therefore, the values may be better derived from the simpler relationship for K_7.

This gives a value for the equilibrium in terms of Ca^{++}, HCO_3^{-}, $HPO_4^{=}$, and H^{+}. This value calculated theoretically from known values of the intermediate constants was found to agree well with the experimentally determined value reported elsewhere.[1] The concentration of the ions could be determined from analysis of all the salts in the system and the calculated degree of ionization. In systems in which this is not possible, due to the presence of unknown compounds as in blood serum, the CO_2 from bicarbonates and H_2CO_3, the P from inorganic phosphates and the H^+ must be determined. The only unknown is the Ca^{++} which may then be calculated from the equation

---

[1] Kugelmass, I. N., and Shohl, A. T., The determination of the equilibria involving calcium, hydrogen, carbonate, bicarbonate, primray, secondary and tertiary phosphat ions., *J. Biol. Chem.*, to appear.

$$Ca^{++} = \sqrt{\frac{K.\left[H^{+}\right]}{\left[HCO_3^{-}\right]\left[HPO_4^{=}\right]}}$$

4 (2236)

Cystine metabolism. II.

By C. P. SHERWIN and A. R. ROSE.

[*From the Department of Chemistry, Fordham University, New York City.*]

In our last communication[1] we stressed the data bearing on the equilibrium Cystine ⇄ Cystein and gave a general outline of the program of the investigation of cystine metabolism. The twelve preparations of cystine and cystein therein described have been fed to rabbits and the urines corresponding to them have been analyzed. The analysis of the large mass of data is not yet complete. In this communication we will discuss the fate of cystine and cystein as such and one of the derivatives and compare our results with data of other workers, *e. g.*, Wohlgemuth[2], Lewis and Root[3] and Schmidt and Clark[4]. All the figures have been recast so as to give the increase in sulfur in mg. following the administration of cystine sulfur, as cystine or its derivative.

---

[1] Shiple, G. J., Rose, A. R., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED, 1923, xx, 360.

[2] Wohlgemuth, J. Z., *physiol. Chem.*, 1903, xl, 81.

[3] Lewis, H. B., and Root, L. E., *J. Biol. Chem.*, 1922, i, 303.

[4] Schmidt, C. L. A., and Clark, G. W., *J. Biol. Chem.*, 1922, liii, 193.

TABLE I.

CYSTINE ELIMINATION.

	Tot. S mg.	″SO$_4$ S mg.	RSH S mg.	Tot. S mg.	″SO$_4$ S mg.	RSH S mg.	Tot. S mg.	″SO$_4$ S mg.	RSH S mg.
Wohlgemuth	I			II					
Rabbits									
Av. control days....	155	126	30	158	121	37			
Cystine days	1242	726	516	692	420	272			
Do. less control	622	224	398	376	178	199			
Lag days less cont.	54	40	13	95	42	52			
Cystine S elimin....	675	264	411	471	220	250			
Lewis and Root	II			IV					
Rabbits									
Av. control days....	24	14	10	74	54	14			
Cystine days	110	91	19	109	96	15			
Do. less control	86	77	9	35	42	1			
Lag days less cont.	18	20	—2	7	10	1			
Cystine S elimin...	104	97	7	42	52	2			
Schmidt and Clark	I			II			IV		
Dogs									
Av. control days....	244	137	107	111	34	77	111	73	40
Cystine days	1371	714	656	888	714	174	1084	799	285
Do. less control	1027	577	549	777	680	97	973	726	245
Lag days less cont.	431	96	336	432	230	200	130	114	15
Cystine S elimin...	1458	673	885	1209	910	297	1138	863	261
Rose and Sherwin	I			II			III		
Rabbits									
Av. control days...	106	40	66	66	41	25	66	41	25
Cystine days	401	187	213	512	403	110	291	205	86
Do. less control	159	123	81	314	281	34	159	123	35
Lag days less cont.	29	24	0	0	0	0	65	58	6
Cystine S elimin...	188	107	81	314	281	34	223	182	41

	Wohlgemuth	Lewis and Root	Schmidt and Clark	Sherwin and Rose
Animals used	Rabbits	Rabbits	Dogs	Rabbits
Basal ration	Carrots	Milk and sugar	Milk, fat and crackers	Carrots
Grams of cystine in each dose	3, 5,	0.5, 0.5	7.5, 7.5, 7.0	0.4, 0.4, 0.4
Cystine days	2, 1,	1, 1,	1, 1, 1,	3, 3, 3.

Lewis and Root, exp. LV, cystine given subcutaneously. Schmidt and Clark, exp. IV, a pup, the other two adults. Sherwin and Rose exp. III, cysteinhydrochloride used in place of cystine.

The total sulfur varies with the food and is reasonably constant. It is highest on the carrot diet, about 50 mg. per kilo, and lowest on the milk diet, 8 to 16 mg. per kilo. The sulfur partition of the control periods bears no relation to anything which is at present known. The per cent of total sulfate sulfur in the average control day urines is given as line A, the order of sequence being the same as that in the general table (I).

	W	S and R	S and C	Authors
A	81.0, 76.5	58.5, 73.0	55.0, 30.3, 66.0	55.0, 62.3, 62.0
B	41.0, 35.5	92.0, 48.8	72.3, 60.2, 61.0	88.1, 98.0, 93.0
C	61.0, 47.0	93.2, 100.0	46.2, 75.3, 75.5	57.0, 89.1, 82.0

Arranged in the same way (line B) are the increments in per cent of total sulfur following the administration of cystine, that is the cystine sulfur eliminated. The two sets have about the same range, but are by no means parallel. The 81 per cent of the controls does not come opposite the 93 per cent of the eliminations nor does the 36 per cent from the line of eliminations come opposite the 30 per cent in control. We have no explanation whatever for the first set of figures. They can not be correlated as to diet, species, sex, nor age. The second set bears some relation to the dosage of cystine. Wohlgemuth gave his rabbits 2000 mg. per kilo and got the lowest elimination; the authors gave 133 mg. in three parts and got the largest elimination. The other two are properly intermediate. An increase in sulfate sulfur at the expense of the sulfhydrate sulfur indicates an oxidation of cystine sulfur. The sulfate sulfur in per cent of the cystine sulfur eliminated is arranged in line C. If there is any parallelism between the cystine sulfur eliminated and the oxidation, it is certainly not striking. Apparently a slow absorption facilitates oxidation. Attention should be called to the large differences in retention of the cystine and to the lag of elimination.

The authors assume that the cystine may be attacked in the body at three points of the cystine molecule; the amino, the carboxyl and the sulfhydrate. We have demonstrated[1] that in the organism cystine can change into cystein, and cystein into cystine. This is not only true of cystine but also of its derivatives. We may therefore speak of the sulfhydrate group in cystine in this special connection. If organic radicles are attached at these vulnerable points the attack on cystine may be inhibited and perhaps prevented. Lewis and Root have presented data on phenyluraminocystine which indicate that this hypothesis is tenable. This is supported also by a number of our own experiments. The degree of inhibition is not the same for the different agencies of blocking and by blocking all around the oxidation might almost be considered as totally prevented. To illustrate this matter we will give the data from experiments with phenyluraminocystine and phenyluramino cystein and for comparison some figures from Lewis and Root. It is to be noted in both these tables that in these experiments cystine and cystein act in identical manner.

TABLE II

CYSTINE AND CYSTEIN PHENYLURAMINO SULFUR ELIMINATION.

	Tot. S mg.	″SO$_4$ S mg.	RSH S mg.	Tot. S mg.	″SO$_4$ S mg.	RSH S mg.
Lewis and Root		I			II	
Av. control days	45	35	10	24	14	10
Cystine days	121	55	66	115	38	77
Do. less control	76	20	56	91	24	67
Lag days less control	12	0	12	23	7	16
Cystine S eliminated	88	20	68	114	31	83
Per cent cystine S eliminated: I 65.6, II 87.0.						
Per cent of this S as total ″SO$_4$: I 21.3, II 27.0.						
Sherwin and Rose		I			II	
Av. control days	88	53	35	64	36	29
Cystine days	424	257	168	399	184	215
Do. less control	160	97	63	207	76	128
Lag days less control	9	9	0	40	23	17
Second lag day less control	50	15	35	16	4	12
Cystine S eliminated	219	121	99	263	103	157
Per cent cystine S eliminated: I 69.5, II 79.0.						
Per cent of this as total ″SO$_4$: I 55.0, II 39.0.						

Phenyluraminocystine given by Lewis and Root, 1 gram in one day; by Sherwin and Rose, 0.8 gram on each of three days. The second experiment by Sherwin and Rose is with the cystein derivative.

5 (2237)

A quantitative study of the destruction of vitamin B by heat.

By H. C. SHERMAN and M. R. GROSE.

[*From the Department of Chemistry, Columbia University, New York City.*]

Using the rat growth method with the system of controls and precautions developed by Dr. Edgeworth, Dr. Spohn and the authors, studies were made of the percentage destruction of vitamin B at the temperatures 100°, 110°, 120°, 130° C when in each case the heating was continued for 4 hours and the vitamin was contained in a water solution (tomato juice) at the natural acidity of the tomato juice, namely pH = 4.3.

The juice of canned tomatoes was chosen as a suitable form in which to study vitamin B and for the further reason that the results here obtained would thus become directly comparable with the results of the studies, previously made in our laboratory, upon the heat destruction of vitamin C.

For descriptions of methods and for experimental data and their discussion, reference must be made to the original papers in the Journal of the American Chemical Society.[1] In the paper of which the present communication is an abstract the effects of different amounts of vitamin B upon the weight curve of the young rat are shown quantitatively and the percentage of vitamin destroyed by heat at each temperature is computed from the experimental determination of the quantity of heated juice which shows the same vitamin value as a given standard quantity (usually 4 cc. per rat per day) of the unheated juice. It is also explained that the percentage destruction computed from the experimental data may differ slightly according as we accept as coincident those results which approximate each other within close limits of experimental error (method A) or "correct" such results by computing what change in the quantities of juices fed would have brought the final points of the weight curves into exact theoretical coincidence (method B). These two methods of interpreting the experimental data yield respectively the following results:

Percentage of vitamin B destroyed in 4 hours heating at pH = 4.3 at temperatures stated—

	100°	110°	120°	130°
Method A	20	33	47	55
Method B	24	33	45	58

---

[1] Sherman, H. C., LaMer, V. K., and Campbell, H. L. The quantitative determination of antiscorbutic vitamin (vitamin C). *J. Am. Chem. Soc.* 1922, xliv, 165.

LaMer, V. K., Campbell, H. L., and Sherman, H. C. The effect of temperature and the concentration of hydrogen ions upon the rate of destruction of antiscorbutic vitamin (vitamin C). *J. Am. Chem. Soc.* 1922, xliv, 172.

Sherman, H. C., and Edgeworth, H. Experiments with two methods for the study of vitamin B. *J. Am. Chem. Soc.* 1923, xlv, 2712.

Sherman, H. C., and Spohn, A. A critical investigation and an application of the rat growth method for the study of vitamin B. *J. Am. Chem. Soc.* 1923, xlv, 2719.

Sherman, H. C., and Grose, M. R. A quantitative study of the destruction of vitamin B by heat. *J. Am. Chem. Soc.* 1923, xlv, 2728.

If from these data we compute the temperature coefficient for the rate of heat destruction through each temperature interval of 10° C we obtain:

	Method A	Method B
Q_{10} (100°—110°)	33/20 = 1.6	33/24 = 1.4
Q_{10} (110°—120°)	47/33 = 1.4	45/33 = 1.3
Q_{10} (120°—130°)	55/47 = 1.2	58/45 = 1.3
Q_{10} (Avg. 100°—130°)	1.4	1.33

In whichever way interpreted the results plainly show that vitamin B is very much more stable than vitamin C; but that the heat destruction of vitamin B does proceed at a measurable rate at 100° C and at an increasing rate as the temperature is raised. The effect of increased temperature upon the rate of heat destruction of vitamin B is (as was also found for vitamin C) less than in the case of most chemical reactions. A rise of 10° C in temperature increased the rate of heat destruction of vitamin B in slightly acid water solution only 1.3 to 1.4-fold, whereas most chemical reactions are increased about 2-fold.

Our experiments give no confirmation to the conclusion of earlier workers that there is a rapid rise in the rate of heat destruction in the neighborhood of 120° C. We find no evidence of departure from the orderly course of a chemical reaction under the accelerating influence of heat; but do find a less than average temperature coefficient.

In respect to its low temperature coefficient the heat destruction of the vitamin is in marked contrast with the heat coagulation of typical proteins and with the heat destruction of such typical enzymes as have been investigated.

6 (2238)

Studies on the purification of antibodies.

By REUBEN OTTENBERG.

[*From the Pathological Laboratory, Mt. Sinai Hospital, New York City.*]

It has been known for years that when precipitates are produced in serum by salts of the heavy metals, immune bodies present are more or less completely precipitated. Whether the immune bodies are precipitated by the metal directly or whether merely adsorbed by the rather voluminous protein precipitate formed has never been determined. It seemed a possibility that the metal might actually enter into combination with the immune body, and that by repeated precipitation of such a combination, purification of the immune body might be brought about. In preliminary experiments with anti-sheep hemolysin and typhoid agglutinin, using the chlorides or sulfates of copper, nickel, zinc, mercury and lead as precipitants, it was found that a good deal of these antibodies could be dissolved out of the precipitates. On account of the large amount of protein present, however, it was found that any really effectual purification of the antibody was impossible.

It was determined therefore, to try the effect of metallic salts on antibody already partially freed of protein by the method of specific absorption followed by subsequent dissociation from appropriate cellular antigens. This we have done both with anti-sheep hemolysin and with typhoid agglutinin. But on account of the interference of the metal salts with the action of complement in hemolysin experiments, the work has been more successful with bacterial agglutinin.

With typhoid agglutinin the preliminary purification was brought about by first sensitizing large amounts of formol-killed typhoid bacilli as nearly as possible to saturation with agglutinin, then washing the sensitized bacteria free of serum in repeated changes of saline solution, and then dissociating the agglutinin from the bacteria either with water at 55° C for one half to one hour, or with N/200 NaOH at room temperature for five minutes. The extracts contained on the average about 0.15 per cent solid matter of which about 0.03 per cent was ash. Their titre ranged from 1/300 to 1/1200. More than half of the solid matter present was undoubtedly derived from the bacteria themselves as was found when control extracts were made of bacteria which had not been sensitized.

When to such extracts metallic salts were added, (copper, nickel, zinc, platinum and mercuric chlorides) precipitates formed, but only when the solutions were brought to a certain hydrogen ion concentration zone. The limits of this zone can not yet be exactly defined; it is not the same for the different metals. The maximum precipitation was usually near pH 6.4. When the amount of metal added was not excessive (not over M/200 strength) the precipitate contained not only nearly all the agglutinin but nearly all the metal present. When these precipitates were redissolved in very weak (N/200) HCl, they were found to contain most of the agglutinin. In order to reprecipitate the agglutinin from this solution again it was only necessary to bring this solution again to the proper hydrogen ion concentration. It was not necessary to add further metallic salt. We have been able to reprecipitate and redissolve such metal-antibody combinations as many as five times in succession with only a slight loss of agglutinin each time.

The specificity of the agglutinin effect and the absence of agglutinination by either the metallic salts, or the acid, or both together, were carefully controlled.

In the case of copper, a second zone of precipitation of the agglutinin was found far to the alkaline side. The hydrogen ion concentration was beyond pH 9.6 and could not be precisely measured by the colorimetric system of Clark, which we employed. This alkaline copper precipitate brings down agglutinin almost quantitatively and is deep blue in color, whereas the precipitate obtained in the nearly neutral zone is pale blue. Like the neutral precipitation the alkaline precipitation can be brought about repeatedly with only slight loss of agglutinin. It seems probable that the precipitate brought down in the alkaline zone is composed partly of copper hydroxide because copper hydroxide can be precipitated in about the same zone by the addition of suitable amounts of alkali to copper chloride solution.

When the control extracts were made from bacteria which had never been sensitized, and treated in the same way (with copper only), a similar looking precipitate, only smaller in amount, was formed in the "neutral" precipitation zone. Just as in the earlier experiments with serum, therefore, the question is again open as to whether the precipitation of agglutinin is here brought about merely by adsorption to precipitated bacterial protein, or whether a separate combination of antibody and metal exists. The almost quantitative recovery of the antibodies after repeated precipitation, however, offers promise that the latter may be the case. If so, since each precipitation seems to leave some of the extraneous bacterial substance behind, this method of repeated precipitation may afford the possibility of ultimate purification of the antibody. Further work is being done in this direction.

7 (2239)

On the nature of the action of vegetable extracts on the blood sugar of normal rabbits.

By HARRY E. DUBIN and H. B. CORBITT.

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The recent work of Winter and Smith[1], Best and Scott[2], Funk and Corbitt[3], and of Collip[4] establishes the point that there is present in yeast and in plants and vegetables a substance which, upon injection into normal rabbits, causes a fall in blood-sugar. In this connection, the experiments of Thalhimer and Perry,[5] and Fetzer[6] led to similar observations.

Best and Scott, working with extracts of potatoes and rice, obtained results like those following an injection of insulin. On the other hand, Funk and Corbitt (unpublished data), as well as Collip, observed from their experiments with various vegetable extracts that there was usually a preliminary hyperglycemia and that the blood-sugar reducing effect did not manifest itself until some time after the injection. They found also that the effect was more prolonged than was the case with insulin.

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[1] Winter, L. B., and Smith, W. J., *J. Physiol.* 1922, lvii, 100.

[2] Best, C. H., and Scott, D. A., *J. Metabol. Research,* 1923, iii, 177.

[3] Funk, Casimir, and Corbitt, H. B., *Proc. Soc. Exp. Biol. and Med.* 1923, xx, 422.

[4] Collip, J. B., *J. Biol. Chem.* 1923, lvi, 513; 1923, lvii, 65.

[5] Thalhimer, W., and Perry, M. C., *J. Am. Med. Assoc.*, 1923, lxxx, 1614.

[6] Fetzer, L. W., *J. Am. Med. Assoc.*, 1923, lxxxi, 772.

Because of this delayed and prolonged action, the above investigators conclude that the blood-sugar reducing substance present in vegetables—"Glucokinin," as Collip calls it—is not identical with insulin.

The results of the present work tend to show that the blood-sugar reducing substance obtained from vegetable sources, if freed from the hyperglycemia-producing principle, behaves in a manner similar to that exhibited by insulin. Detailed data will shortly be published elsewhere; for the present, we may summarize briefly as follows:

1. It appears that yeasts and vegetables such as cabbage, celery, lettuce, etc., contain both blood-sugar increasing and blood-sugar decreasing substances.

2. Crude extracts, when injected, may produce either a rise in blood-sugar or a rise followed by a fall below the normal.

3. The same effect may be produced by the injection of *l*-suprarenin bitartrate (synthetic).

4. In one experiment, 6 cc. of whole blood of a normal rabbit injected into another normal rabbit resulted in a 20 per cent decrease in blood-sugar in 54 hours. Two other experiments gave doubtful results. These experiments were undertaken to satisfy ourselves as to the effect of the injection of normal blood. They are not intended to disprove Collip's statement as to the animal passage of a hypoglycemia-producing substance.

5. On fractionating crude extracts, it is possible to separate almost entirely the hyperglycemic from the hypoglycemic substance.

6. The preliminary fractionation is effected with alcohol; the final separation involves the use of dinitrosalicylic or picric acid. Working with pancreas, Dudley[7] also used picric acid for this purpose.

7. The hypoglycemic substance from vegetables may be ad-

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[7] Dudley, H. W., *Biochem. J.*, 1923, xvii, 376.

sorbed by charcoal, as was pointed out in work on pancreas extracts by Best and Macleod[8] and by Murlin, Clough, Gibbs and Stokes.[9] In a number of cases, it has been possible to extract the hypoglycemic substance from charcoal (norit) with glacial acetic acid.

8. The hypoglycemic substance, when injected, gives results identical with those produced by insulin.

9. The delayed and prolonged action of vegetable extracts described by other investigators may be due to the inhibiting effect of the blood-sugar increasing substance.

10. It may be mentioned, in passing, that the mere ingestion of glucose, galactose, fructose or starch produces a rise in blood sugar, followed by a fall below normal, as was shown by Liefmann and Stern,[10] Frank,[11] McLean and deWesselow,[12] and Foster.[13]

In the light of the foregoing summary it is evident that the blood-sugar reducing substance present in various plants and vegetables, when freed from the blood-sugar increasing substance and injected into normal rabbits, produces a fall in blood-sugar typical of that caused by an injection of insulin.

This observation necessarily militates against the theory of the existence of a new hormone, "Glucokinin," in plant tissue, particularly in view of the fact that it is possible to duplicate the "delayed and prolonged" blood-sugar reducing action by the injection of a number of widely separated and unrelated substances.

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[8] Best, C. H., and Macleod, J. J. R., Proceedings, *J. Biol. Chem.*, 1923, lv, p. xxxix.

[9] Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stokes, A. M., *J. Biol. Chem.*, 1923, lvi, 253.

[10] Liefmann, E., and Stern, R., *Biochem. Z.*, 1906, i, 299.

[11] Frank, E., *Z. physiol. Chem.*, 1910-11, lxx, 291.

[12] McLean, H., and de Wesselow, O. L. V., *Quart. J. Med.*, 1921, xiv, 103.

[13] Foster, G. L., *J. Biol. Chem.*, 1923, lv, 291 and 303.

8 (2240)

Non specific immunity to diphtheria and tetanus toxins not induced.

By WILLIAM N. BERG.

[*From the Berg Biological Laboratory, Brooklyn, N. Y.*]

Guinea pigs were injected subcutaneously with a number of antigens with the expectation that non specific as well as specific immunity would be induced. The non specific immunity was looked for by testing the guinea pigs' resistance to diphtheria and tetanus toxins and to several pathogenic organisms.

Suspensions of pure proteins (casein, edestin) and dead organisms *(B. coli, B. pyocyaneus)* were the antigens used. They were prepared as follows: a 1.000 gm. portion of pure dry protein was suspended in 20 cc. of 0.5 per cent acid sodium phosphate solution. The suspensions contained in sterile weighing bottle were incubated, tested for sterility, and when found free from contaminating organisms were diluted with 0.8 per cent sodium chloride. The calculated volume of protein suspension was then injected. Acid sodium phosphate solution was used for suspending the antigens in order to avoid disintegration or autolysis of protein with attendant formation of toxic products as suggested by Thomson.[1]

Cultures of *B. coli and B. pyocyaneus* were grown 18 to 24 hours on 1 per cent dextrose 3 per cent agar slants without peptone. Peptone was omitted because Hitchens[2] found that organisms grown on peptone agar are much more toxic than those grown on peptone free media. The growths were suspended in a solution containing 0.5 per cent phenol, 0.5 per cent chloroform and 0.5 per cent acid sodium phosphate. No heat was used to render bacterial suspensions sterile. These were diluted with 0.8 per cent sodium chloride solution just before subcutaneous injection. The weight of dry organisms injected was determined by drying a portion to constant weight, and making the necessary corrections.

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[1] Thomson, D., *Lancet*, 1919, June 28, p. 1102.

[2] Hitchens, A. P., *Interstate Med. J.*, 1914, xxi, 537.

No attempt was made at bacterial counts. An approximate idea of the number of organisms injected was obtained from the data of Wilson and Dickson.[3] According to these investigators one milligram of dry *B. coli* contains 6.5 billions; one milligram of dry *B. pyocyaneus,* 3.5 billions of organisms.

The diphtheria and tetanus toxins and antitoxins used were the standard preparations furnished by the Hygienic Laboratory, Washington, D. C. One minimal lethal dose of Diphtheria Toxin No. 266 for 500-600 gm. guinea pigs was 0.015 cc. injected subcutaneously. One minimal lethal dose of Tetanus Toxin No. F 3 for 350 gm. guinea pigs was 0.0075 mg. subcutaneously.

Guinea pigs received as many as three injections of the same antigen. The doses were as large as was consistent with safety. That it was safe was judged by the weights of the experimental animals throughout the tests. A week or more after the last injection of antigen, the resistance of the guinea pigs to diphtheria and tetanus toxins was tested by injection of one to three minimal lethal doses. In none of the animals was the slightest non specific resistance detected—all died in the same time as the controls. Thus Guinea Pig No. 53 received three injections of dead *B. coli.* These organisms were chloroform killed, but not otherwise altered nor dried. The organisms in the doses injected weighed, 5, 10 and 10 mg. when dried. This did not protect the animal against a little more than one minimal lethal dose of tetanus toxin. (See Tables I and II.)

TABLE I.

NON RESISTANCE OF IMMUNE GUINEA PIGS TO TETANUS TOXIN.

Guinea Pig No.	Initial weight	Final weight	Number of minimal lethal doses of toxin injected	Time of death
	gm.	gm.	April 7	hours
8	—	585	control 2	88
3	—	645	" 3	64
5	—	540	" 2	88
4	—	640	" 3	88
			April 19 May 24	76
			GLYCININ mg.	
19	590	545	25 3	
22	570	555	25 3	67

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[3] Wilson, W. J., and Dickson, Ch., *J. Hyg.*, 1912, xii, 49.

			April 21	April 28	May 4	May 24	
			CASEIN mg.				
30	530	540	5	7.5	10	3	67
36	560	575	5	7.5	10	3	92
			May 2	May 7	May 14	May 24	
			EDESTIN mg.				
41	570	575	10	15	15	3	67
47	520	550	10	15	15	3	69
			May 6	May 12	May 26	June 13	
			B. COLI dead mg.				
53	520	595	5	10	10	3	65
61	630	620	5	10	10	3	65
			May 17	May 25	June 6	June 13	
			B. PYOCYANEUS dead mg.				
65	600	605	5	5	10	3	65
70	600	605	5	5	10	3	73
86	—	545	control			2	65
87	—	635	"			3	65

TABLE II.

NON RESISTANCE OF IMMUNE GUINEA PIGS TO DIPHTHERIA TOXIN.

Guinea Pig. No.	Initial weight	Final weight	Number of minimal lethal doses of toxin injected				Time of death
	gm.	gm.				April 7	hours
2	—	565	control			1	64
7	—	580	"			1.3	64
6	—	565	"			1	64
1	—	515	"			1.3	44
			April 19			May 10	
			GLYCININ mg.				
10	505	510	25			1	46
13	690	645	25			1	66
15	500	495	25			1	50
			April 21	April 28	May 4	May 10	
			CASEIN mg.				
27	605	605	5	7.5	10	1	75
28	540	535	5	7.5	10	1	66
29	595	545	5	7.5	10	1	66
63	—	465	control			1	66
64	—	515	"			1	92
65x	—	550	"			2	66
			May 6	May 12	May 26	June 13	
			B. COLI dead mg.				
55	640	610	5	10	10	1	73
62	620	595	5	10	10	1	116
			May 17	May 25	June 6	June 13	
			B. PYOCYANEUS dead mg.				
69	670	675	5	5	10	1	70
74	595	605	5	5	10	1	90
88	—	675	control			0.7	116
89	—	620	"			1	90

The initial weight is weight immediately before first injection of antigen. Final weight is weight immediately before toxin injection. Weights were carefully watched to make certain that resistance to toxin was measured only in apparently normal healthy animals.

The above results are similar to those obtained by Cowie and Kempton[4] and by Kolle and Schlossberger.[5] They found that injecting guinea pigs with typhoid organisms or horse serum did not protect against small doses of diphtheria toxin, (3 mld.). That three guinea pigs receiving 100 times the fatal dose of diphtheria toxin were saved with normal horse serum as reported by Kastenmeyer,[6] is extremely doubtful.

Data on the non specific resistance of guinea pigs to several organisms are omitted. The difficulty of accurately determining the minimal lethal dose of a live culture obscured the results. Non specific agglutinins were looked for but not found. The methods of calculating and preparing the doses of standard antitoxins, together with several useful modifications of the standard or Hygienic Laboratory technic have already been described by Berg.[7]

9 (2241)

The antiketogenic influence of insulin in diabetes.

By JOHN A. KILLIAN.

[*From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York.*]

The object of this study was to determine the action of insulin upon the production and excretion of ketone bodies and upon the acid-base equilibrium of the blood of human cases of diabetes mellitus. This report presents the results of seventeen observations upon fourteen patients. The only therapeutic agent employed was insulin.

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[4] Cowie, D. M., and Kempton, R. M., *J. Med. Research*, 1921, xlii, 227.

[5] Kolle, W., and Schlossberger, H., *Med. Klin.*, 1919, l, 83.

[6] Kastenmeyer, B., *Deutsch. med. Woch.*, 1919, xlv, 1338.

[7] Berg, W. N., *J. Infect. Dis.*, 1921. xxix, 86.

To avoid the necessity of utilizing other agents such as alkalies or glucose, it was essential to grade the dose of the insulin so as to secure the maximum beneficial effect without producing untoward results. In a series of preliminary experiments it was demonstrated that for individuals weighing from 50 to 70 kg. one unit of insulin decreased the blood sugar about 0.008 per cent in from 4 to 6 hours. The doses of insulin employed in the present studies were calculated as the quantity required to decrease the initial blood sugar to 0.130 per cent.

Previous to the administration of the insulin the patients had been starved overnight for 12-14 hours, and during the period of observation lasting from 8 to 10 hours, no food was taken. However, water by mouth was permitted *ad libidum*. As a control on the factor of starvation in 5 cases, two series of curves were obtained representing changes in the blood sugar and carbon dioxide combining power of the plasma with and without insulin. It was noted that the starvation alone continuously decreased the blood sugar about 0.04 per cent, but this drop is insignificant in comparison with the fall after insulin. In three instances the carbon dioxide combining power rose steadily during starvation, but in the 2 remaining cases a fall of 5 vol. per cent was noted.

The insulin used in these experiments was the product of the Eli Lilly Co. The subjects were all patients of Dr. H. O. Mosenthal of the Department of Medicine. Before the administration of the insulin a control specimen of blood was obtained, and a specimen of urine collected over one or two hour periods. After the subcutaneous injection of the insulin, specimens of blood and urine were secured every hour or two hours. The sugar, ketone bodies and carbon dioxide combining power of the blood were determined, and in the urine the concentration and the actual excretion in grams per hour of the sugar and ketone bodies were studied. The total ketone bodies were estimated as acetone in the blood and urine by Van Slyke's gravimetric method. The specimens of blood were analyzed immediately after withdrawal by venipuncture to obviate any source of error due to glucolysis or the production of fixed acid bodies in the blood upon standing.

In three cases analyses were confined to the blood since it was impossible to obtain simultaneous urine collections from these patients who at the outset were in a coma. It was observed that following the administration of the insulin the blood sugar decreased, the decline reaching the maximum 4 to 6 hours. The total fall in blood sugar was from 50 to 60 per cent of the control. The lowest figure obtained was 0.104 per cent. The drop in blood sugar was accompanied by a corresponding diminution of the urine sugar, both in per cent and in grams per hour.

The rise in the carbon dioxide combining power was very striking, from 8 to 20 vol. per cent. The highest values obtained were noted 4 to 6 hours after the insulin. All of the cases studied showed in the control specimens of blood a marked increase in the concentration of ketone bodies in the blood and urine. After the insulin a decrease to 40 to 16 per cent of the original concentration in the blood was found, the fall closely paralleling the rise in the carbon dioxide combining power. A corresponding drop in the excretion of the ketone bodies of the urine was also noted. A rise of the blood and urine sugar and of the blood and urine ketone bodies and a decrease of the carbon dioxide combining power of the plasma were observed 8 to 10 hours after the insulin injection.

In six instances determinations of the blood pH were made by the Myers' method simultaneously with the carbon dioxide combining power or content of the blood. A rise in the pH was observed corresponding to the rise in the combining power or of the content of carbon dioxide. It is evident from the results obtained that in diabetes mellitus insulin inhibits for a short period the production of ketone bodies, and in this manner readjusts the acid-base equilibrium of the blood.

10 (2242)

The direct determination of secondary phosphate.

By I. NEWTON KUGELMASS and CARMEN ROTHWELL.
(by invitation).

[*From the Department of Pediatrics, Yale University, New Haven, Conn.*]

Saturated calcium sulfate added to a solution of primary and secondary phosphates precipitates the latter according to the equation

$$4Na_2HPO_4 + 3CaSO_4 \rightleftarrows Ca_3(PO_4)_2 + 2NaH_2PO_4 + 3Na_2SO_4.$$

The phosphorus of the secondary phosphate is equally distributed between the tricalcium phosphate and the monosodium phosphate. The primary phosphate does not precipitate and hence no primary phosphate present would be transformed into secondary phosphate.

Analysis of the precipitate thus obtained showed that it was $Ca_3(PO_4)_2$. Maximum precipitation occurs when the HPO_4 and $CaSO_4$ are in the molal ratio of 4:3 as determined by conductivity measurements. This establishes the equation above. Under these conditions the reaction gives 93 per cent precipitation. Completion can be attained when the $CaSO_4$ is present in five times the concentration of the HPO_4.

Data by the method published elsewhere[1] show that in the ratio of 1:19 or 19:1 primary phosphate does not interfere with the determination of secondary phosphate. If a simultaneous determination of the total phosphate is made, the ratio of primary and secondary phosphates and hence the hydrion concentration can be calculated.

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[1] Kugelmass, I. N., and Rothwell, C., The direct determination of the secondary phosphate, *J. Biol. Chem.*, to appear.

11 (2243)

Studies on the peritoneal fluid in anaphylactic shock.

By MOYER S. FLEISHER and LEO L. MAYER.

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In recent years stress has been laid in particular upon changes in the physical and chemical reactions of the body fluids in anaphylactic shock and it has become evident that more or less profound changes can be demonstrated. A number of years ago Segale[1] investigated changes of the osmotic pressure of the blood in shocked animals and found that in dogs, rabbits and guinea pigs there occurred a marked fall of the freezing point of the serum during shock, which fall lasted over a considerable period of time—even twenty-four hours. As we were unable to find in literature any confirmation of these observations we undertook, at least in the case of the guinea pig, to confirm Segale's results.

We used animals sensitized to egg white and reinjected them intraperitoneally after three weeks or more. At the same time that the sensitized animals received the second injection, we injected normal animals with an equal amount of egg white. The animals were bled at periods varying from 15 minutes to 5 hours after the injections and the freezing point of the serum was determined. The average Δ of the 32 normal animals was —0.578° while that of 34 readings of shocked animals was —0.574°. It appears, therefore, that Segale's observations are not correct, at least as far as concerns the guinea pigs.

We thought it might be of interest to determine whether we could find any difference between the Δ of fluid which had been injected into the peritoneal cavity of normal and of shocked animals. Again we used animals sensitized to egg white; gave a second injection three to six weeks after the first injection, consisting of 6 cc. of a 50 per cent egg white intraperitoneally and also 30 cc. of sodium chloride solution. The animals were killed at periods varying from 12 to 15′ up to 120′ after injection; the abdominal cavity opened and the fluid collected. At the same time normal animals were injected intraperitoneally with 6 cc. of a 50 per cent egg white and 30 cc. of sodium chloride solution, and the fluid collected after similar periods.

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[1] Segale, *Pathologica*, 1911, iii, 323; 1912, iv, 12.

The average of the results of the determinations of the freezing point are given in Table I.

TABLE I.

Sensitized Animals			Control Animals	
Time between injection and recovery of fluid	Δ	Number of animals	Δ	Number of animals
15′	—0.562°	129	—0.551°	117
30′	—0.586°	21	—0.571°	18
60′	—0.581°	14	—0.558°	16
120′	—0.565°	16	—0.540°	18
All Periods	—0.566°	180	—0.553°	167

It is apparent from the above table that the fluid in the peritoneal cavity of the shocked animals shows at all periods a more marked lowering of Δ than that of the control animals; that this phenomenon is not a brief and momentary reaction, but that it is still marked at one and two hours after the shocking injection; and that possibly there is a tapering-off at the two hour period.

It is evident then, that absorption of materials from or movement into the peritoneal cavity in shocked animals is not the same as in normal animals. It is possible that the difference in the freezing point might be due to a difference in the absorption of the sodium chloride in the two series of animals. The following table shows the average results of the determination of chlorides in the recovered fluids.

TABLE II.

Shocked Animals			Control Animals	
	NaCl—mg.	Number of animals	NaCl—mg.	Number of animals
30′ series	795	14	800	15
All series	757	95	748	96

The slight differences noted here are certainly within the limits of experimental error, and do not account for the differences in freezing point in the two series.

We do not find that there is any difference in the amounts of fluid absorbed in the two series—of the total of 36 cc. of fluid injected in the series in which thirty minutes or more elapsed between injection and removal of the fluid, we recovered an average of 25.7 cc. of fluid from 32 shocked animals, and 25.3 cc. of fluid in 38 control animals.

Since in our experiments we can exclude the possible factor of changed osmotic pressure of the serum in shocked animals as an influence upon the osmotic pressure of the fluid introduced into the peritoneal cavity, we believe that these experiments give what seems to be direct evidence that in anaphylactic shock the permeability of cells or cell membranes is definitely affected.

At the present time then, while it is probable that in animals in anaphylactic shock there is a changed activity of the permeability of the cells or cell membrane (that is, the peritoneum) we cannot state whether this change is in relation to organic or inorganic substance, to crystalloids or to colloids. Certainly the changed permeability does not affect the sodium chloride, and apparently from a limited number of experiments it does not affect the phosphates. It seems, therefore, more likely that permeability in relation to certain organic substances, either in dissolved or colloid state, may be affected.

12 (2244)

A note on the antigenic properties of the filtrate of typhoid saline vaccine.

By WILLIAM A. PERLZWEIG, LOIS WELLS and HAROLD L. AMOSS.

[*From the Chemical and Biological Divisions, Department of Medicine, The Johns Hopkins University and Hospital, Baltimore, Md.*]

It was recently shown by Perlzweig and Steffen[1] that the immunizing antigen of the pneumococcus may be extracted from the bacterial cells by 70 per cent alcohol, physiological salt solution and by water. They found that the supernatant fluid of old pneumococcus saline vaccines when filtered through a Berkfeld candle conferred active immunity upon mice after several subcutaneous injections. Such filtrates also produced type-specific precipitates with homologous immune sera. The agglutinin production was not studied.

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[1] Perlzweig, W. A., and Steffen, G. I., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 378, and *J. Exp. Med.*, 1923, xxxviii, 163.

In view of these findings experiments were begun on the antigenic properties of the supernatant fluids of other vaccines. In a preliminary way the following was performed with typhoid vaccine:

The contents of a number of 0.5 and 1 cc. vials of a commercial triple typhoid (T. A. B.) saline vaccine, date of expiration February 13, 1923, bacterial content 1,000 million typhoid and 750 million of each paratyphoid A and B, were thoroughly mixed by shaking, a total volume of 30 cc. being collected. 15 cc. of this mixture were removed and are designated below as "whole vaccine". The remaining 15 cc. were centrifugalized at high speed until the supernatant fluid was clear. This fluid was carefully decanted and filtered through a Berkfeld (N) candle. Two rabbits were then injected intravenously with the whole vaccine and two with the filtrate, and the agglutinin and precipitin titres of the rabbit sera determined.

The dosage and titres are tabulated below:

	Rabbits immunized with whole vaccine.					Rabbits immunized with filtrate of supernatant fluid.				
Date	Dose	Aggl't'n titre		Precipitin titre		Dose	Aggl't'n titre		Precipitin titre	
1923	injected	Rb.A.	Rb.B.	Rb.A.	Rb.B.	injected	Rb.C	Rb.D.	Rb.C.	Rb.D.
Apr. 18	bleeding	0	0	0	0	bleeding	0	0	0	0
" 18	0.5 cc.					0.5 cc.				
" 25	1.0 cc.					1.0 cc.				
May 2	1.0 cc.					1.0 cc.				
" 9	bleeding	1:1280	1:5120			bleeding	1:640	1:640		
" 15	1.0 cc.					1.0 cc.				
" 22	1.0 cc.					1.0 cc.				
" 29	bleeding	1:1280	1:2560	1:160	1:160	bleeding	1:320	1:2560	1:160	1:160

It is evident from these figures that it is possible to obtain in the sera of rabbits immunized with the Berkfeld filtrate of a typhoid saline vaccine almost as high agglutinin and precipitin titres as in the sera of rabbits treated with the whole vaccine. This shows that at least two of the antigens, the agglutinogen and preciptinogen, of the typhoid bacillus are soluble in and can be extracted by dilute salt solution. That these antigens are in all probability distinct from the higher protein constituents of the bacterial cell may be deduced from the assumption that such proteins were coagulated by the heating of the vaccine in the process of manufacture and also by the fact that the supernatant filtrate used in this experiment gave a barely perceptible biuret reaction, and it contained only 0.5 mg. of nitrogen per 100 cc. as compared with 29.3 mg. nitrogen per 100 cc. of the whole vaccine. A more extensive immunological and chemical study of the water soluble antigen of the typhoid bacillus will be reported at a later date.

After the described experiment had been performed the interesting study by Nichols and Stimmel[2] appeared reporting the production in guinea pigs of some active immunity against infection with the "mutton" strain of *B. aertrycke* by injections of the supernatant fluid of T. A. B. vaccine. This finding lends further support to the theory that the immunizing agent of bacterial vaccines is a water soluble substance which is simpler in character than the proteins of the cell.

13 (2245)

Toxicity of atropin on rats.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

According to most textbooks of Pharmacology, the rat is very resistant to the effects of atropin. Wilberg[1] in an original monograph on the subject of the toxicity of atropin for various animals states that on subcutaneous injection the fatal dose of atropin for rats is 0.75 gm. per kilo weight while a dose of 0.70 gm. per kilo may be followed by recovery. On intraperitoneal injection the same author states that the fatal dose for rats is

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[2] Nichols, H. J., and Stimmel, C. O., *J. Exp. Med.*, 1923, xxxviii, 283.

[1] Wilberg, *Biochem. Z.*, 1914, lxvi, 389.

0.6 gm. per kilo weight while the animal may recover from 0.5 gm. per kilo. In connection with experiments on the belladonna alkaloids on the behavior of rats, published elsewhere, the author found that rats are extremely sensitive to atropin so far as toxic effects on the brain are concerned. Thus it was found that 1 mg. of atropin sulfate was very toxic for a rat weighing 150 gm. as indicated by its behavior in the circular maze and its neuromuscular responses. It was found that the smallest dose of atropin sulfate which produced a deterioration in the cerebro-spinal responses of rats was 0.05 mg. per 150 gm. weight of animal. It is thus evident that while the absolute lethal dose of atropin for rats may be very great as stated by other authors, the dose required to impair certain physiological functions is very small. Fuller details appear elsewhere.[1]

14 (2246)

The feeding of odd carbon fatty acid fats to diabetic patients.

By MAX KAHN.

[*From the Department of Laboratories, Beth Israel Hospital, New York City.*]

This artificially prepared fat (glyceryl ester of margaric acid) is absorbed to the extent of ninety-five per cent. If a psychologic distaste for it is not induced in the patient by the physician (who thinks it his duty to warn the patient of the supposed non-palatability of the fat), the patient will have little trouble in partaking of this artificial food. An experience with very many patients has convinced the author that all patients can easily develop a liking for this food. It may be administered with hot coffee or rubbed up with butter milk.

It has been found that the administration of this fat to phlorhizinized dogs causes a marked increase in the glucose elimination in the urine, showing that *in the dog* it is broken down to propionic acid and is then converted to glucose, according to the theory of Ringer and his collaborators.

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[1] Macht, D. I., *J. Pharm. and Exp. Ther.*, 1923, xxii, 25.

To those individuals on whom insulin appears to have no effect, or to those patients who refuse insulin, or to patients on whom insulin produces a moderate effect, the odd carbon fat may be safely administered in portions that yield as high as 1,000 calories daily. If, for example, a patient's diet has been increased by means of insulin to an intake of 1500 calories daily, we can add 1000 calories to his diet by means of this fat and thus establish an approach to a maintenance diet.

Moderately severe cases of diabetes who altogether refuse insulin medication, or become discouraged with this medication after a period of months, may be kept in normal weight and their appetite sated by the administration in liberal amounts of this "odd carbon" fat.

The accompanying table shows the effect of feeding this "odd carbon" fat to a diabetic and tubercular individual.

TABLE I.

Date	Food				Urine		Blood	
	P.	C.	F.	Odd Fat	Glucose	Ketones	Glucose	CO_2
2/20	40	20	42	0	Traces	++	0.224	44
2/22	40	35	40		1.4 per cent	++	0.227	46
2/24	37	13	31	0	0	0	0.185	48
3/2	40	20	42	0	Traces	++	0.195	42
3/6	37	13	31	0	0	0	0.175	48
3/10	42	15	37	0	0	0	0.162	48
3/14	42	15	37	100	0	0	0.151	54
3/20	42	15	37	100	0	0	0.148	54
5/24	42	15	37	150	0	0	0.113	58
6/2	44	17	37	150	0	0	0.134	58

15 (2247)

The behavior of inorganic phosphate in the blood and urine of normal and diabetic subjects during carbohydrate metabolism.

By WILLIAM A. PERLZWEIG, EMILY LATHAM and
CHESTER S. KEEFER (by invitation).

[*From the Chemical Division, Medical Clinic, Johns Hopkins Hospital, Baltimore, Md.*]

Harrop and Benedict[1] and Wigglesworth, Woodrow, Smith and Winter[2] have recently called attention to the fall of the concentration of the inorganic phosphate in the blood of human subjects and experimental animals following the use of insulin. In the course of various studies on diabetes, particularly in connection with the studies on the respiratory exchange of normal and diabetic subjects following the ingestion of glucose, glycerol, calcium hexose phosphate and calcium glycerophosphate[3] and the respiratory exchange following the administration of insulin and epinephrin[4] data were accumulated on the changes of the inorganic phosphate in the blood and urine under the above enumerated experimental conditions. It was found that in normal human subjects the ingestion of 50-100 gm. of glucose and the intravenous injection of 3.5 units of insulin, also the subcutaneous injection of 0.5 cc. of 1:1000 solution of epinephrin caused a marked fall in the inorganic phosphate of the blood, and a simultaneous fall of the rate of excretion of phosphate in the urine. With the injection of epinephrin, however, in normal subjects the fall of the blood phosphate was frequently accompanied by a rise in the rate of urinary excretion of phosphate. The excretion of phosphate was most singularly depressed by the ingestion of glycerol. While in all of the cases studied this retention of phosphate was accompanied by a rise of the respiratory quotient, indicating increased carbohydrate metabolism, with the ingestion of glycerol the marked retention of phosphate was accompanied by a lowered respiratory quotient.

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[1] Harrop, G. A., and Benedict, E. M., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 430.

[2] Wigglesworth, V. B., Woodrow, C. E., Smith, W., and Winter, L. B., *J. Physiol.*, 1923, lvii, 447.

[3] McCann, W. S., and Hannon, R. R., *The Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 73, and McCann, W. S., Hannon, R. R., Perlzweig, W. A., and Tompkins, E. H., *Arch. Int. Med.*, 1923, xxxii, 226.

[4] Lyman, R. S., Nichols, E., and McCann, W. S., *J. Pharm. and Exp. Therap.*, 1923, xxi, 343.

With diabetic subjects it was frequently observed that the ingestion of glucose or the injection of insulin did not cause a retention of phosphate especially when the blood sugar curves and the respiratory exchange indicated that there was no increased catabolism of carbohydrate. No general relationship was found between the retention of phosphate and the reaction of the urine as measured by the total titratable acidity. The determinations of reducing sugar and of phosphate in blood filtrates before and after hydrolysis with acid failed to reveal the presence in the blood of a carbohydrate-phosphate compound at the height of phosphate retention. If such compounds as hexose phosphate are formed in the process of the oxidation of carbohydrates they are probably to be found in the liver and muscles.

Our data furnish obviously further support to the theory of the close participation of the phosphate ion in the metabolism of carbohydrates.

16 (2248)

The oxygen consumption of thyroid and diiodotyrosine-fed tadpoles.

By O. M. HELFF (by invitation).

[*From the Osborn Zoological Laboratory, Yale University, New Haven, Conn.*]

It has been known for some years that thyroid-fed tadpoles undergo precocious metamorphosis, and more recently the fact has been demonstrated that diiodotyrosine has a similar effect upon amphibian larvæ. Students of amphibian metamorphosis have assumed that an increase in metabolic rate occurs during transformation, and the statement is even made that metamorphosis is due to increased metabolism. So far as the writer is aware, however, no attempt has been made to test the validity of these assumptions. It seemed worth while, therefore, to measure the metabolic rate of precociously metamorphosing tadpoles at various stages of transformation. Since oxygen consumption is considered a rough gauge of metabolic rate, this method was employed.

EXPERIMENTAL

During the months of June, July, August and September, 1923, two hundred *Rana pipiens* tadpoles of approximately the same size and developmental stage were collected. They were fed for two days on Spirogyra and then starved for an equal period, preparatory to running the initial test for oxygen consumption. Dessicated thyroid, containing .20 per cent of iodine, and diiodotyrosine were the agents employed to accelerate metamorphosis. The animals were placed in small jars of water, one tadpole to a container, and kept at a constant temperature of 22 ± 1° C. It was found that continued feedings of thyroid lowered the resistance of the animals to such an extent that death occurred before advanced stages of metamorphosis were reached. Consequently, the method employed was to feed the tadpoles one or two days with thyroid and then keep the animals in solutions of diiodotyrosine which, although it greatly hastens metamorphosis, does not weaken the larvæ to the extent that is true of continuous administrations of thyroid.

Successive stages of metamorphosis were arbitrarily selected: the normal tadpole showing no indications of transformation was designated stage one, the metamorphosed animal whose left fore leg had appeared and been free for at least a day was designated stage 8. The remaining six stages represented intermediate points of development. On the average, a thyroid-diiodotyrosine-fed tadpole passed from stage 1 to stage 8 in a period varying from eight to eleven days. Tadpoles so metamorphosed and whose oxygen consumption had been measured several times, invariably died after reaching stage 8, and consequently, no measurements were made on animals with both fore legs through the operculum.

The Winkler method was used throughout in determining the oxygen consumption, the water used in the experimental flasks being in all cases maintained at 24° C. It was found necessary to etherize tadpoles of stage 1 (normal animals) during the test in order to eliminate errors arising from the activity of the individuals under operation. Etherization was found unnecessary in all succeeding stages since tadpoles undergoing transformation remain quiescent for considerable periods. All individuals were weighed accurately to the milligram, whenever tested, in order to determine the oxygen consumption per gram weight.

RESULTS

The results obtained show, beyond doubt, that there is a progressive increase in oxygen consumption per gram weight of metamorphosing tadpoles. Between stages 1 and 2 there is an increase of 48 per cent. Stages 3 and 4 show a still further increase of 59 and 76 per cent respectively. The next two stages (5 and 6) show a somewhat lessened increase over stage 1 as compared with stage 4 or 61 and 54 per cent respectively. The last two stages (7 and 8) give a respective increase of 71 and 79 per cent over the normal or stage 1 consumption, stage 8, as was expected owing to the advanced stage of metamorphosis, possessing the greatest increase per gram weight of any stage tested. Table I gives the average actual amount of oxygen in cubic centimeters consumed per gram weight in one hour for the eight stages, while Fig. 1 represents graphically the per cent increase in oxygen consumption of the same individuals.

TABLE I.

Average Oxygen Consumption in cc. per gm. wt.

No. Tested	STAGE								Av. Increase
	1	2	3	4	5	6	7	8	
32	.157	.233							.076
19	.160		.254						.094
33	.160			.282					.122
36	.178				.286				.108
16	.171					.264			.093
27	.174						.298		.124
10	.192							.344	.152

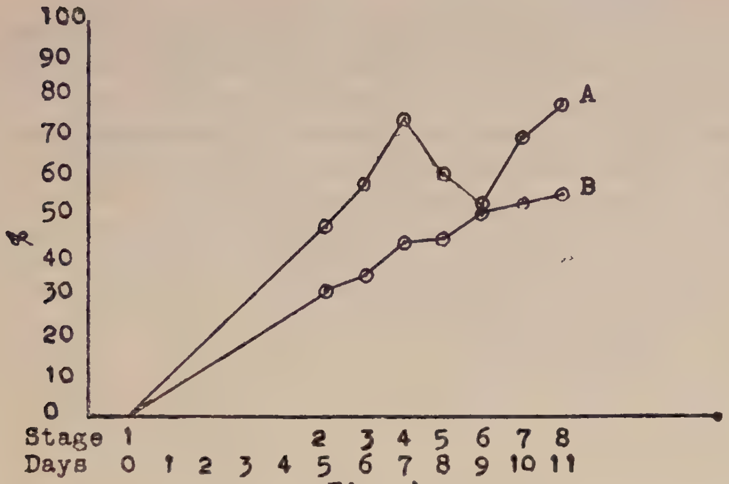

Fig. 1.

A. Av. % increase oxygen consumption per gm. wt.
B. Av. % decrease in total weight.

By referring to Table II, it is at once evident that there is a progressive decrease in total weight as the tadpole attains each successive stage of metamorphosis. Fig. 1 emphasizes this fact by graphically representing the per cent decrease in total weight as compared with the normal or stage 1 weights of the same individuals. If the per cent increase of oxygen consumption per gram weight was of the same percentage as the per cent decrease in total weight at any given stage, the conclusion would be that no increase in total oxygen consumption had occurred. In reality, however, the data show a progressive increase in total oxygen consumption with an increasing stage of metamorphosis.

TABLE II.
Average Weight in gms.

No. Tested	STAGE								Av. Decrease
	1	2	3	4	5	6	7	8	
32	1.394	.942							.452
19	1.451		.922						.529
33	1.409			.793					.616
36	1.398				.765				.633
16	1.375					.662			.713
27	1.400						.643		.757
10	1.415							.605	.810

The bulk of the increase per gram weight, however, is accounted for by the large decrease in total weight. The falling off of the per cent increase in oxygen consumption in stages 5 and 6 is in a large measure due to the fact that many of the tadpoles tested at this stage were in a weakened condition and died within five to ten hours after the test and before reaching stage 6 or 7. In other words, the critical period in thyroid-diiodotyrosine metamorphosis seems to be during these stages. Once past these stages, the tadpoles recovered their vigor and hence the increased oxygen consumption in stages 7 and 8.

The effect of etherization showed that, if anything, anesthetization by this agent increased oxygen consumption. In fact, the average increase per gram weight of sixteen individuals so anesthetized, amounted to 19 per cent, which also represents the per cent increase of total oxygen consumed, since the normal and etherized tests were run within an hour of each other during which period no reduction in weight had occurred. This fact, then, adds emphasis to the percentage increases of oxygen consumption recorded in the later stages.

DISCUSSION

The writer was fortunate in obtaining a full series of tadpoles of the same species used in the oxygen tests, which were undergoing normal metamorphosis. Several striking dissimilarities were noticeable when compared to a similar series of thyroid-diiodotyrosin metamorphosed tadpoles. The greatest difference was found in the atrophy of the tail as compared to the growth of the limbs. For example: a normally metamorphosing tadpole, which had succeeded in protruding the left fore limb through the operculum, possessed a tail in the same stage of atrophy as was to be found on a typical stage 4 thyroid-diiodoytrosine metamorphosed tadpole, where the left fore limb would require several days yet to appear. In other words, the fore limb appeared much earlier in relation to the atrophy of the tail in the normal series than in the thyroid-diiodotyrosine series.

Other such differences in correlation were observed, making it clear that the thyroid-diiodotyrosine type of metamorphosis did not permit the atrophy, autolysis or growth of certain parts to proceed in the normal manner. This undoubtedly accounts for the high mortality rate met with in all series of animals so metamorphosed. It would also account, in a large measure, for the considerable variation in oxygen consumption of individuals of the same metamorphic stage. This variation in oxygen consumption made it necessary to base the consumption at any given stage on the average of a considerable number of animals.

CONCLUSIONS

1. *Rana pipiens* tadpoles, metamorphosed with dessicated thyroid-diiodotyrosine exhibit a progressive rise in metabolic rate as evidenced by oxygen consumption per gram weight. In stage 8 the metabolic rate reaches a maximum increase over that of stage 1 of approximately 79 per cent.
2. Precociously metamorphosed tadpoles increase their total oxygen consumption regardless of decreasing weight.
3. Thyroid-diiodotyrosine-fed tadpoles undergo a gradual decrease in weight amounting to 57 per cent in stage 8.
4. Etherization of stage 1 tadpoles (normal animals) results in approximately 19 per cent increased oxygen consumption.

17 (2249)

The study of hemolytic streptococci associated with scarlet fever.

By FRANKLIN A. STEVENS and A. R. DOCHEZ.

[*From the Department of Medicine of Columbia University, College of Physicians and Surgeons, and the Presbyterian Hospital, New York City.*]

The present work was undertaken subsequent to the work of Gordon, Bliss and of Tunnicliff in the study of hemolytic streptococci associated with scarlet fever. The object of the work was to study the agglutination of streptococci from the throats of patients with scarlet fever, and to study the phenomenon of agglutinin absorption with these bacteria. The strains were gathered from numerous sources. The technique was similar to that of Bliss.[1]

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[1] Bliss, W. P., *J. Exp. Med.*, 1922, xxxvi, 575.

The first part of the work consisted in a survey of scarlatinal patients designed to determine the percentage of agglutinable strains in acute cases of scarlet fever, and to determine the number of patients who had agglutinable streptococci in their throats at the end of the quarantine period. Forty acute cases were included in the first group. Hemolytic streptococci were isolated from 87.5 per cent of these throat cultures. 74.3 per cent of the strains isolated from these acute cases were agglutinated by immune sera prepared with two scarlatinal strains. Among the convalescents, streptococci were found in 47.3 per cent. 55.0 per cent of these strains could be agglutinated with scarlet immune sera.

The second part of the work consisted in cross agglutination reactions between strains obtained from scarlet fever occurring in several large cities of the United States and Europe. A series of strains obtained from a milk borne epidemic of scarlet fever, both from the throats and from the suspected milk, were included in the series. The strength of the reaction varied with the various combination of sera and strains, but in the main the cross agglutinations were consistent and strains obtained from conditions other than scarlet fever were not agglutinated.

The absorption of agglutinin was carried out at 55° C. Fresh immune sera and strongly agglutinable strains were necessary for agglutinin absorption. All the combinations of sera and strains did not give absorption but each strain was represented in the absorption experiments, since absorption of agglutinin from several immune sera was accomplished with each strain, or immune serum prepared with a given strain was absorbed by several other scarlet strains.

Since the strains represent cases of scarlet fever occurring in New York City, Chicago, Baltimore, San Francisco, and Copenhagen, Denmark, as well as cases occurring during a milk borne epidemic, it is to be concluded that the strains of hemolytic streptococci associated with scarlet fever are closely related biologically, and probably constitute a single unit group.

Abstract of Communications.

Peking Branch.

Third meeting.

Peking, China, May 16, 1923.

18 (2250)

The protective action of gelatin for pneumococci in suspension.

By O. H. ROBERTSON, RICHARD H. P. SIA, and SHUTAI T. WOO.

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

In order to carry out certain experiments in which very small numbers of pneumococci were to be used, it became necessary first to determine the type of non-nutrient fluid most suitable for preserving suspensions of pneumococci with a minimum amount of injury. For comparative studies, dilutions in the various fluids tested were made from a standard suspension containing approximately 1000 million pneumococci per cc. The preservative properties of each solution were judged by the length of time viable organisms could be recovered (in culture) from that dilution containing 0.000,000,1 cc. of the standard suspension. The hydrogen-ion concentration of the solutions, the temperature at which the suspensions were kept, and the culture media were carefully controlled. Furthermore, plates were made at the beginning of the experiment to determine the number of organisms present in a unit of suspension.

It was found that pneumococci suspended in 0.9 per cent NaCl solution, Locke's solution and water remained alive for only a few hours at most. Salt solution was shown to be the least suitable of these three; not infrequently by the time the 0.000,000,1 cc. dilution in this fluid had been reached, the suspension was sterile. The addition, however, of 0.1 per cent gelatin to any of the above fluids transformed them into highly favorable solutions for the suspension of this organism. Pneumococci suspended and diluted in gelatin-water and gelatin-

Locke's solution remained alive at room temperature for 6 to 7 days; in gelatin-salt for 2 days. Series of plates made at frequent intervals failed to reveal any growth in solutions containing this small concentration of gelatin. The beneficial effect of gelatin was found to lie largely in its protection of the pneumococci against mechanical injury, which occurs during the process of dilution in crystalloid solutions or water. Gelatin has in addition a well marked preservative action, the nature of which is uncertain.

ABSTRACT OF COMMUNICATIONS.

Western New York Branch.

Eighth meeting.

Clifton Springs, New York, October 13, 1923.

19 (2251)

Some experiments on the excretion of ammonia.

By ROGER S. HUBBARD and ELLERY G. ALLEN.

[*From the Laboratories of the Clifton Springs Sanitarium, Clifton Springs, New York.*]

In a previous article Hubbard and Munford[1] published the results of a study of the ammonia excretion in a series of cases; in the present article a similar study of a number of determinations upon a single subject is given. Determinations of the reaction and ammonia content were made upon samples of urine collected at hourly intervals through the morning from a normal man (one of the authors, E. G. A.). Experiments were carried out on thirteen days during a period of two and a half months during the summer of 1923. The food eaten for breakfast varied on the different days, but otherwise there was no noteworthy difference between the various experiments. Analyses were carried out as described in the previous paper.[1]

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[1] Hubbard, R. S., and Munford, S. A., The excretion of acid and ammonia. *J. Biol. Chem.*, 1922, liv, 465.

TABLE I.

Independent Variable			Dependent Variable		
Kind	Variation	Pairs	Volume	Ammonia Nitrogen	
			per cent	milligrams	
				per cc. per cent	per hr. per cent
Reaction	any	1743	69	82	64
"	more than 0.2 pH	1422	75	87	67
"	" " 0.5 pH	1019	80	93	71
"	" " 1.0 pH	536	89	98.5	80
Volume	any	1768		75	51
"	more than 5 cc.	1342		81	49
"	" " 10 cc.	995		86	49
"	" " 20 cc.	491		89	53

Ammonia concentration and rate of excretion increase as degree of acidity increases. Volume decreases as degree of acidity increases. Rate of ammonia excretion increases and concentration of ammonia decreases as volume increases.

In the first table the results of the experiments have been summarized as were those in the paper by Hubbard and Munford. All the results were listed in the order of the differences in reaction and volume, all the pairs of values of amounts of ammonia excreted and of concentrations of ammonia which varied in the same direction as the independent variable were counted, and the result expressed as per cent of the total pairs. Similar calculations have been given for specimens which varied from each other by more than 0.2 pH, by more than 5 cc., etc. The table shows that the volume and the concentration and rate of excretion of ammonia varied with the reactions of the specimens, and that the ammonia concentration varied much more closely with it than did the rate at which ammonia was excreted. These results agree with the findings given in the first article. Ammonia excretion did not vary with the volume as it did in the earlier series. Specimens with large volumes were usually excreted in the middle of the forenoon when the degree of acidity was low, and this fact may partially explain the lack of agreement. The ammonia concentration varied with the volume, but no more closely than the volume itself varied with the reaction, and not as closely as the ammonia concentration varied with the reaction.

TABLE II.
Reaction varies 0.2 pH or less.

Volume varies	Pairs	Ammonia per hour varies with volume
	Number	
more than 1 cc.	410	71 per cent
" " 5 cc.	273	76 "
" " 10 cc.	166	77 "
" " 20 cc.	67	91 "

In Table II the relationship between volume and ammonia excretion of all pairs of specimens whose reactions did not differ from each other by more than 0.2 pH is given. The rate of ammonia excretion did vary with the volume in these specimens, but not as closely as it did in the series previously reported. Most of the specimens with constant reactions in that series were found in cases of gastric anacidity in which the reaction did not vary during the whole experiment; it seems possible that the difference in the agreement in the series now presented arises from changes in reaction which took place during the periods of collection.

TABLE III.
Average values of each experiment.

Reaction	Volume	Ammonia Nitrogen	
		per 100 cc.	per hour
pH	cc.	mg.	mg.
5.2	20.5	69	14
5.2	28	70	20
5.5	23	66	14
5.8	37	48	17.5
5.9	27	58.5	16
6.0	36	61	8
6.0	38	35	13
6.1	40	40	14
6.15	46	40.5	16.5
6.15	41	54	22
6.5	35	32	11
6.7	36.5	32	10
6.85	47	28	12.5

The average of the results of each day, arranged in order of differences in reaction, are given in Table III. The ammonia concentration varied with the reaction (there was only one pair of experiments which differed from each other by more than 0.2 pH which is not in its proper sequence) but the agreement is not quantitative. This failure of quantitative agreement was also shown in the tables from which Table I was compiled. The rate of ammonia excretion did not correspond with the reaction as it did when separate specimens were compared with each other.

TABLE IV.

Experiments with small variations in reactions of specimens.

Reactions of specimens		Volume	Ammonia Nitrogen
Difference pH	Average pH	Average cc. per hour	Average mg. per hour
0.2	5.2	20.5	14
0.4	5.5	23	14
0.2	5.2	28	22
0.4	5.65	18	13.5
0.4	5.45	34	22
0.5	4.9	36	14
0.3	4.8	39	15

In the first part of Table IV the average of those experiments in which the reactions of the different specimens was fairly constant and the average reactions of different experiments agreed well is given. Since only three experiments in the series fulfilled these conditions, results on two other subjects are also given. In these experiments the average rate of ammonia excretion varied with the volume.

The results show that in this series the ammonia concentration varied with the reaction, and that when the reaction was constant the rate of ammonia excretion varied fairly closely with the volume. It is possible that variations in the rate of ammonia excretion may depend on some factor which varies approximately with the volume and that this may explain why better agreement between volume and ammonia excretion was not found.

20 (2252)

Effect of thyroidectomy upon the reaction of sheep to insulin.

By AARON BODANSKY (by invitation).

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

The author has suggested previously that the effect of thyroxin upon glycogenolysis could be demonstrated after the synthesis of glycogen brought about by the administration of insulin, and submitted some experimental evidence.[1]

Further evidence of the glycogenolytic function of the thyroid is yielded by data upon the reaction of normal and thyroidectomized sheep to the intravenous administration of a standard dose of insulin. In normal sheep a regular drop in the sugar content of the blood takes place, the sugar values reaching a minimum within *about* 35 *minutes* after the injection. Recovery to normal sugar values begins immediately, being complete within 1½ to 2 hours after the injection.

In thyroidectomized sheep the decrease of the sugar values continues until *about* 50 *minutes* after the injection and a more intense hypoglycemia is produced. Recovery to the initial sugar values proceeds at a strikingly slower rate than in normal sheep.

It is suggested that the thyroid is essential to normal recovery from insulin hypoglycemia. Experiments are proceeding upon clinical applications of this principle.

This work was carried out under a grant from the Heckscher Research Foundation.

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[1] Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 538.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF COMMUNICATIONS.

One hundred thirty-fourth meeting.

Rockefeller Institute for Medical Research, November 21, 1923.
President Jackson in the chair.

21 (2253)

A study of the anaphylactogenic properties of aqueous extracts of pollens and Le Page's glue.

By MATTHEW WALZER and ELLA F. GROVE (by invitation).

[*From Cornell University Medical College, and the New York Hospital, New York City.*]

A study, with the Dale method, of a series of isolated guinea pig uteri, from animals injected with extracts of timothy and giant ragweed pollens, confirmed the findings of Parker that pollen extracts are antigenic. The uterine horns were found to be specifically sensitive only to the pollens with which the animal had been injected. This specificity exists even for pollens generically closely related; in other words, all attempts to obtain contractions in uteri sensitized with giant ragweed pollen by the use of low ragweed pollen extract as the antigen were unsuccessful.

Intravenous injections of the specific concentrated antigen in sensitized guinea pigs failed to kill any of the animals by acute anaphylactic shock. Precipitins could not be demonstrated in any of the animals' sera, nor have attempts at passive transfer of the sensitivity as yet met with success.

Le Page's Glue has antigenic properties very similar to those of pollen extracts.

The characteristic feature, which differentiates this group of antigens from the known anaphylactogens, is their ability to elicit second, third, fourth, and more contractions from the same sensitive horn, by the repeated addition of the *same* amount of antigen to a fresh bath. This failure to conform with the quantitative rules governing the successive partial neutralization of precipitins, as demonstrated by Coca and Kosakai, and confirmed by the authors in the anaphylactic guinea pig uterus, together with the failure to demonstrate precipitins and passive transfer of sensitivity in this group of animals, makes it problematical whether we can include these antigen-antibody reactions under the heading of anaphylaxis as we understand that term at present.

22 (2254)

The nature of the pollen atopen.

By ELLA F. GROVE and ARTHUR F. COCA.

[*From Cornell University Medical College, and the New York Hospital, New York City.*]

It has been assumed that the exciting agent of atopic coryza and asthma in pollen is a protein.

We were at first inclined to the belief that this agent was a non-dialyzable protein, on account of the observation that, although about two-thirds of the nitrogen of the pollen extract could be eliminated by dialysis, the atopic activity of the extract remained unaltered.

This belief was proved to be incorrect by the further observation that, after digestion of the protein with trypsin, and thorough dialysis, the pollen extract was found to be just as active as before this treatment, although no trace of its original nitrogen content remained in it.

This result seems to place the pollen atopen in the general category of the enzymes, without, however, in any way demonstrating that the substance possesses the function of an enzyme.

23 (2255)

A study of the atopic reagin.

By ARTHUR F. COCA and ELLA F. GROVE.

[*From Cornell University Medical College, and the New York Hospital, New York City.*]

The mechanism of atopic hypersensitiveness has been demonstrated in the blood by Ramirez, deBesche, and Prausnitz and Küstner, by the method of passive transfer.

This reacting substance in the blood of atopic individuals (reagin) readily attaches itself to the cells of the normal recipient, remaining active for at least sixteen days. The skin of some normal individuals is non-receptive, that is, it cannot be passively sensitized with atopic serum. This difference in normal skins bears no relation to the difference among human individuals in their susceptibility to poison ivy.

The atopic reagin is not destroyed by exposure to a temperature of 56° C. for one-half hour.

Local passive sensitization is effected in normal human beings without incubation period. The mixture of the serum of an atopic individual and its related atopen produces an immediate reaction when injected intradermally into the normal human skin, and is toxic for some normal guinea pigs upon intravenous injection.

The symptoms produced by such injection into guinea pigs are not those of anaphylaxis.

The atopic reagin can be completely neutralized with the related atopen. There seems to be no factor in the successive partial neutralization of the atopic reagin, as there is in the successive partial neutralization of precipitin.

The atopen of *Ambrosia Trifida* has been found to be identical with that of *Ambrosia Artimisiæfolia*.

Sensitiveness to poison ivy could not be transferred even with serum of an exquisitely sensitive individual.

Sensitiveness to tuberculin could not be transferred to the skin of non-sensitive human individuals with the serum of a tuberculin sensitive individual.

24 (2256)

The penetration of trivalent and pentavalent arsenic into living and dead cells.

By MATILDA MOLDENHAUER BROOKS.

[*From the Division of Pharmacology, Hygienic Laboratory, Washington, D. C.*]

The biological effects of trivalent and pentavalent arsenic are not the same. The former are not readily excreted by the body and are more toxic; the latter are readily excreted and are less toxic. It is obviously not possible to analyze the microscopic cell contents of body-cells to note how much arsenic is taken up by each cell. By making use of a large one-celled marine alga, *Valonia,* whose sap can be expressed readily and analyzed for arsenic content, it was thought that some conclusions of general application to living protoplasm might be found. It was possible to separate the plant into the three components; sap, protoplasm, and wall, and analyze each one for arsenic. The ratio of the weights of each component was also found; the sap being 165 times heavier than the protoplasm and 257±13 times heavier than the wall.

All the experiments were done in sea water. Arsenic in the form of As_2O_5, As_2O_3, and atoxyl was used in a concentration of .002 M. The H ion concentration was varied in two ways: by using buffers and by the addition of traces of acid. The presence of arsenic was determined by the Gutzeit method. All the reagents used were special arsenic-free. The plants remained 20 hours in the arsenic solution. They were not injured by this treatment.

It was very important to ascertain whether the plants were in good condition after the experiment. This was done by returning them to sea water after they had been in the test solution and noting the number of days they survived as compared with normal cells. Other superficial methods of observation were found ~~not~~ sufficient.

The results showed that by far the greatest concentration of arsenic is taken up by the protoplasm. The pentavalent form of arsenic is taken up and retained in the greatest concentration by the protoplasm and in a much lower concentration by the sap; when the trivalent form is used, there is much less arsenic found in the protoplasm and about twice as much in the sap. From acid solutions of the trivalent form the amount of arsenic which penetrates into the protoplasm is considerably reduced. The minimum amount of arsenic is found in the sap and protoplasm when the solutions are nearly neutral. Under all conditions the concentration of arsenic in the wall is less than that in the protoplasm, and exceeds that in the sap.

When phosphate buffers (0.0028 M) are dissolved in the surrounding solution in the acid range together with the arsenic, there is a reduction in the rate of penetration of arsenic. This effect is least when trivalent arsenic is used. It is very necessary therefore in experiments in which buffers are used to know the effect of the buffer ions themselves.

Living and dead cells were placed in solutions in sea water containing enough arsenic acid to produce a pH of 3.6. Time curves show that the penetration into dead cells is much more rapid than into living cells.

It was shown in a previous paper[1] that when $NaHCO_3$ is present in the surrounding solution the sap has more free CO_2 and at the same time becomes richer in basic ions than in sea water of the same pH without $NaHCO_3$. Therefore it was thought of interest to see whether other substances such as arsenic, which can be readily detected, would also enter more readily under the influence of $NaHCO_3$. Cells were placed for one hour in a .0024 M solution of $NaHCO_3$ in sea water before exposure to arsenic. It was found that this treatment affected the rate of arsenic penetration in a marked way: when As_2O_5 was used the amount of arsenic in the protoplasm was increased 150 per cent more and that in the sap 25 per cent; with As_2O_3 about 25 per cent less of arsenic was found in the protoplasm and 100 per cent more in the sap. The amount of arsenic in the walls was not changed.

These results show that the increased HCO_3 or free CO_2 in the cell either facilitates the formation of arsenic compounds with protoplasm, or increases the permeability of the cell.

Details of this article will appear in Public Health Reports.

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[1] Brooks, M. M., U. S. Public Health Reports, 1923, xxxviii, 1470.

25 (2257)

The reaction system of luminescence in Mnemiopsis.

By A. R. MOORE.

[*From the Department of Physiology, Rutgers College, New Brunswick, N. J.*]

In dark adapted *Mnemiopsis* luminescence occurs upon stimulation such as gentle agitation of the vessel containing the animal. If agitation is continued the light keeps up for a few seconds and then ceases. After a few minutes' rest the reaction may be obtained again. If rested animals are exposed to light of sufficient intensity and duration they cease to luminesce when stimulated. Hence the luminescence reaction may be represented by the following scheme:

$$D \quad \underset{\text{dark}}{\overset{\overset{2}{\text{illumination}}}{\rightleftarrows}} \quad A \quad \underset{\text{rest}}{\overset{\overset{1}{\text{stimulation}}}{\rightleftarrows}} \quad L$$

In which A represents the luminescent substance in the resting, dark adapted state, L the light-giving compound, and D the decomposition product of A caused by illumination. The "rest" reaction of 1 proceeds during illumination and the "dark" reaction of 2 is facilitated by stimulation. The relation of light intensity to the time of exposure required to suppress luminescence is given by the equation $I \times t = K$ where I is the intensity in candle meters, t is time in minutes and K is a constant.

Luminescent paper may be prepared from *Mnemiopsis* in the fashion described by Heymans and Moore for *Pelagia*. Such papers glow in isosmotic solutions of KCl, K_2SO_4, $CaCl_2$, $MgSO_4$ but do not glow in solutions of $MgCl_2$, $NaCl$, and Na_3 citrate. Variations in pH value of the solutions between 6.0 and 8.0 do not appreciably affect the reaction. Illumination of the indicator paper causes no deterioration in its luminescent properties. Therefore in suppressing luminescence in the animal, illumination acts not on the luminescent substance directly but upon some other structure of the organism, presumably a sense organ. No glow is elicitable from the paper by cytolytic agents such as solutions of alcohol, ether, or butyric acid in sea water, or sea water warmed to 40° C., and hence luminescence in this case does not depend upon cytolysis.

26 (2258)

The locus of the action of veratrin in Mnemiopsis.

By CHARLES WIBLE.

[*From the Department of Physiology, Rutgers College, New Brunswick, N. J.*]

Specimens of *Mnemiopsis* immersed in a solution of veratrin (1 : 1345) in sea water show immediate inhibition of beats of the swimming plates, these stopping in the upright position. If, now, single plates or groups of plates be isolated from a specimen, in two minutes these begin beating and continue to do so for half an hour or longer in the solution of veratrin. With intact specimens the plates begin to show movement at the tips, only after fifteen minutes' immersion in veratrin. This movement increases in speed and regularity, becoming abnormally vigorous at the end of an hour. At this stage, during momentary cessation of beat, it can be seen that the plates stop in the upright position, and that mechanical stimulation causes, not inhibition of the beat and assumption of the prone position as in the normal animal, but excitation and acceleration of beat. This abnormal type of beat persists for six hours, appearing in pieces of the animal after disintegration.

Since the initial effect of veratrin, namely, inhibition of beat, can be removed by isolation of plates and groups of plates from the animal, and since in the secondary stage of veratrin poisoning, an inhibition is converted into an excitation, it seems probable that the locus of veratrin action in *Mnemiopsis* is on the nerve net.

27 (2259)

Phototropism in Cerianthus.

By MARY MITCHELL MOORE.

[*From the Department of Physiology, Rutgers College, New Brunswick, N. J.*]

Cerianthus membranaceus is an actinian which is positively heliotropic. It is always oriented with its oral disk and tentacles toward the source of light whether the light be diffuse daylight, or artificial light in a dark room. Direct sunlight, however, causes it to retract into the sand. Measurements were made of the amount of light necessary to cause heliotropic bending. The relation between the time of exposure necessary and the intensity of the light follows the equation expressing the Bunsen-Roscoe law, namely, $K = I \times t$. I = intensity, t = time in seconds, K = constant.

Measurements were made of the total reaction time taken between the instant when the beam of light was thrown across the animal and the instant when the ends of the tentacles began to move. The equation expressing this relation is of the same type, $K = I(t + p)$, in which p is a constant.

In case *Cerianthus* is acted upon by two lights of unequal intensities, the relation between the angle turned (α) and the ratio of the intensities of the lights is expressed by the equation $\log I_1/I_2 = K \tan \alpha$, where $K = .615$. This means that the muscle tonus on a given side is proportional to the log I on that side. Therefore, since the angle turned is the result of muscle tonus, the tangent is proportional to the logarithm of the ratio of the two intensities.

28 (2260)

Reflex immobility and the central nervous system.

By W. J. CROZIER.

[*From the Zoölogical Laboratory, Rutgers College, New Brunswick, N. J.*]

A thoroughgoing theory of animal conduct must be prepared to deal successfully not merely with the orienting movements of animals, as Loeb's tropism conception and the work related thereto seems capable of doing; it must also be in a position to treat the problem of quiescence at one moment, movement at another, and in general the whole matter of "physiological states" in which the central nervous system functions as adjustor organ. The inducible tonic immobility markedly developed among arthropods, often termed "feigning of death" and commonly referred to as an "instinct," provides opportunity for study of just this kind of central nervous function.

The length of an act of immobility initiated by appropriate stimulation of an insect or crustacean is in reality determined by the duration of a sustained condition in the central nervous system. It is easily shown that in a variety of Hexapods and Isopods with which these experiments have been concerned, the animal gives other well defined reflexes without emergence from the specific immobile state. With *Ranatra* in particular several striking proofs are obtained of the reciprocal innervation of the appendages and of the orientations of their central connections.

Nevertheless, as Raboud has insisted, in each organism there are certain peripheral loci stimulation of which at once arouses an immobile specimen at the will of the experimenter.

The duration of successive acts of immobility is rhythmic. In Isopods at least, the rhythm does not exist as a general metabolic rhythm on which the observations are, so to speak, superimposed. It is initiated by the first stimulations of a series. Once initiated, however, a cycle rises to a maximum of possible duration, then falls to practically zero duration, independently of further excitations. This is proved by results of tests in which various known intervals elapse between acts of immobilization; and by tests in which the animal is aroused to normal activity before its spontaneous "emergence," then re-immobilized.

The stimulus evoking tonic reflex immobility thus serves apparently to release a system of events, possibly synaptic in location, which determines at any moment the duration of a then initiated control of motor elements by suitable stimulation. The nature of this fundamental cycle, of quite appreciable duration, is deducible from the form of the cycle of successive immobilizations.

This view of central nervous processes is rather different from that customarily entertained. It has obvious advantages for the explanation of such general central phenomena as "facilitation," "reinforcement," and "after discharge."

29 (2261)

On the character of central nervous processes.

By W. J. CROZIER and H. FEDERIGHI.

[*From the Zoölogical Laboratory, Rutgers College, New Brunswick, N. J.*]

Upon what basis central nervous functions operate has been even more mysterious than the mechanism of nerve-fiber conduction. A large rôle is assignable to the synaptic junction, and to its changes in resistance. Attempts to show that memory and forgetting, favorable to analysis because of their duration and gradual change, are really due to the modification of materials in the cortex, have not been altogether convincing. The development and decay of speed in the execution of a simple action has been described (Robertson) with the aid of the formula of autocatalysis, but it can scarcely be held that this result demonstrates the autocatalytic transformation of a synaptic material concerned in central transmission.

The reflex immobility of certain arthropods, and particularly of the isopod *Cylisticus convexus*, is found to be of rhythmic duration. If suitably stimulated following spontaneous emergence from induced immobility, the isopod again becomes immobile, but for a longer time; the duration of later acts of immobility decreases. Continuously repeated tests of this sort show a periodic rise to a certain maximum duration. The amount of this maximum duration is determined chiefly by the temperature. At 6°, the maximum duration is 270 sec.; at 29°, 23 sec. The cycle of immobility is not the reflection of a general metabolic cycle, but is determined by a central nervous process initiated by the first stimulations of the series of trials.

The relation of temperature to the processes underlying the duration of reflex immobility involves comparing the durations at different temperatures but at *corresponding stages of the cycle.* Between 5° and 16° the maxima lie upon one exponential curve with respect to temperature; above 16°, and continuing to 30°, the maxima lie upon an interesting exponential curve of lesser slope. The exponential relation of duration to temperature makes it likely that the duration is determined by an amount of substance produced in a chemical system, rather than that the maxima in the duration curves are to be considered points of minimum "speeds of emergence" from the immobile state. The curves are in fact fitted by Arrhenius' equation,

$$K_2/K_1 = e^{\frac{\mu}{2}\left(\frac{T_2-T_1}{T_2T_1}\right)}$$

with $\mu = 24{,}000$ for the range 5°-16°, $\mu = 9{,}200$ for 16°-30°. These values of μ are within the range characteristic for chemical processes.

It appears, then, that the duration of an act of reflex immobility is determined by a condition similar to synaptic transmissivity continuously maintained for the duration of the act, and itself proportional to the amount of a substance formed by and broken down in two catenary processes having different temperature coefficients.

This general view may be extended to the consideration of "synaptic resistance" in vertebrates. It places certain phenomena of selective transmission in the central nervous system very definitely within the chemical sphere.

30 (2262)

A note on the reaction of protoplasm.

By W. J. CROZIER.

[*From the Zoölogical Laboratory, Rutgers College, New Brunswick, N. J.*]

A variety of considerations lead to the view that the pH of living matter is lower than that of its immediate extra-cellular medium. It is customarily stated, nevertheless, that protoplasm is "neutral or faintly alkaline" in reaction. If this were invariably so, difficulty would arise in understanding the behavior of nervous tissues of insects, for example, where the hemolymph may be acid.

Indirect estimations tend to show that the contents of echinoderm eggs (Vlès) are distinctly acid in reaction. Calculations of the inner pH of nerve have been made (Adams) from the standpoint of the Donnan equilibrium probably concerned in the production of the current of action; but these have involved the assumption that the pH of the fluid bathing the nerve is the same as that of the blood.

The direct determination of intra-cellular acidities is difficult because suitable indicators do not as a rule accumulate within the cell. In some instances, however, dibromthymolsulfonephthalein does serve as a non-toxic indicator of the pH of protoplasm. The staining is diffuse. The cells successfully used included those of *Paramoecium* and other ciliates, in a normal medium of pH 7.3; these gave tints of the indicator suggesting an inner pH distinctly on the acid side of neutrality, corresponding, in fact, to pH 6.7 or a little less. In the case of *Opalina*, the inner pH is lower, 6.2. The reaction of the indicator to alkalies ($NaOH$, NH_4OH) shows that it is not merely condensed on the external surface of the organism.

The nervous tissue of certain insects (larval dipterons) stains similarly. In certain other cases where absorption of the dye is visible, as in cells of the mesenteric epithelium of insects, the protoplasmic pH is also unequivocally on the acid side. This is consistent with information derived from the study of indicator pigments obtained from the cells of various marine animals. It seems possible that protoplasm is in general significantly acid in character, rather than alkaline or neutral.

31 (2263)

Influence of an artificial peroxidase upon the growth of anaerobic bacilli.

By O. T. AVERY and H. J. MORGAN.

[*From the Hospital of The Rockefeller Institute for Medical Research, New York City.*]

Previous study of the influence of plant tissue upon bacterial growth showed that sterile unheated plant tissue can replace blood in the cultivation of the so called hemophilic organism *B. influenzæ.* It was also found that plant tissue exerts a marked accelerating action upon the growth of pneumococcus. More recently it has been shown that the addition of unheated vegetable (potato) to plain bouillon makes possible the continued, ærobic growth of certain anærobic bacilli such as *B. histolyticus, B. ærofetidis, B. oedematiens,* and *B. chauvei.*

This growth promoting action upon three distinct groups of bacteria so widely different in their cultural requirements, indicates that plant tissue meets certain physiological needs of the bacterial cell not wholly provided for by the ordinary culture media. These studies suggest that this action may be dependent not only on the presence of some growth accessory substances in plant tissue but also upon the functioning of certain systems which are concerned in the cellular processes of oxidation and reduction.

McLeod and his associates have found that certain ærobic bacteria which are devoid of catalase form hydrogen peroxide when grown in the presence of air. In the case of pneumococcus, which possesses no catalase, hydrogen peroxide is known to accumulate in the fluid of ærobic cultures in concentrations which are bacteriostatic and even bacteriocidal. As far as is known anærobic bacilli are also devoid of catalase, and hence these cells cannot destroy peroxides. From these relations it seems not unlikely that anærobic bacteria fail to grow in the presence of air, not because atmospheric oxygen as such is a direct poison to the cell, but because of the toxic action of peroxides which may be formed as the result of the union of molecular oxygen with some autoxidizable substance in the bacterial cell. Under these circumstances organisms which are peculiarly sensitive to the action of these peroxides may not only fail to grow but actually die. If this assumption is correct, then the ærobic growth of obligate anærobes in the presence of plant tissue finds partial explanation at least in the fact that peroxides formed are rapidly broken up by the oxidases of the tissue. Under these cultural conditions, therefore, so far as the toxic action of peroxides is concerned, the sensitive cell is protected almost as effectually as though it were growing under anærobic conditions.

Since iron is known to exert an accelerating action upon certain cellular oxidations, and is commonly found in conjunction with the peroxidase of plant tissue, it seemed possible that this substance might function in the oxidative mechanism of the bacterial cell and in the destruction of toxic peroxides in a manner analogous to that of plant tissue. As ferrous sulfate is known to accelerate many oxidation and reduction processes, and exhibits the usual reactions of peroxidase, iron in this form was chosen for study. However, when a solution of ferrous sulfate is added to broth, precipitation occurs. To overcome this, use was made of the method employed by Dony-Hennault in the preparation of artificial laccase. A solution of gum arabic and ferrous sulfate was precipitated in alcohol. The resulting precipitate is soluble in water and in aqueous solution gives the reaction of peroxidase with benzidine and hydrogen peroxide. Solutions of this gum-iron preparation remain stable in bouillon, the gum apparently functioning as a protective colloid. Quantitative analysis of this preparation shows that it contains approximately 20 mg. of iron per gram. In broth containing small amounts of this preparation the obligate anærobes studied were found to grow through repeated transfers in the presence of air.

32 (2264)

The quantitative action of ultraviolet light on Staphylococcus aureus.

By FREDERICK L. GATES.

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By means of a compensated thermocouple of extreme sensitivity kindly made for us by Professor Pfund of Johns Hopkins University, direct measurements have been made of the ultraviolet light energy, at various wave lengths, required to kill *Staphylococcus aureus.* The organisms were exposed on agar plates to the lines of the quartz mercury arc spectrum, isolated by a monochromatic illuminator with quartz lenses and prism. After incubation, counts were made of the colonies produced by surviving organisms in the exposed areas.

Comparison with control counts from adjacent, unexposed, areas shows that the bacteria vary widely in individual susceptibility so that the most resistant may survive an exposure 12 to 20 times that to which the least resistant succumb. The average values of a number of observations at each wave length produce a smooth curve which shows the ratio between the incident energy in ergs per square millimeter and the percentage of organisms killed. The light energy required to kill 50 per cent of the exposed bacteria, at each wave length, has been taken as the mean value of the bactericidal effect of incident ultraviolet light.

Measured thus, as incident energy, wide differences were found in bactericidal power at different wave lengths of the mercury arc spectrum. The lethal energy for 50 per cent of the exposed organisms ranged from 154 ergs per square millimeter at 2378 A.U., through 88 ergs at 2675 A.U, to 3,150 ergs at 3022 A.U. In a single determination the corresponding light energy at 3126 A.U. was 25,000 ergs per square millimeter. Intermediate wave lengths gave intermediate values in an orderly sequence and a line through the observed points produced a curve similar to, but not identical with, that for the absorption of ultraviolet light by *S. aureus* in the same region of the spectrum.

It is only the energy absorbed by the bacteria which can effect a chemical change and the figures for incident energy must therefore be corrected for the percentage of incident light actually absorbed. Measurements of absorption by thin layers of *S. aureus* pressed between quartz plates gave the first values used for this correction. For a layer of organisms one coccus thick the figures for the absorption of light lay between 4.4 per cent at 3022 A.U. and 10.7 per cent at 2535 A.U. When this correction was applied to the observations on incident energy it was found that the absorbed energy involved in the death of *S. aureus* was approximately equal at different wave lengths from 2482 A.U. to 2804 A.U., an interval that corresponds roughly to a broad absorption band of these organisms in the far ultraviolet.

Further consideration suggests, however, that the absorption of ultraviolet light by *S. aureus,* or any cell, is not a function of the entire organism, but the summation of the absorption factors of numerous chemical entities composing its protoplasm. It is necessary to look further for the particular substances that are responsible for light absorption, with the prospect that one or more may be found to be light-sensitive to the degree implied by the bactericidal power of ultraviolet light. Although other protoplasmic constituents may absorb light, they are not necessarily involved in this particular phenomenon. This phase of the subject is at present under investigation.

33 (2265)

A chart showing graphically the respiratory quotient and the percentage of calories furnished by protein, fat and carbohydrate.

By EUGENE F. DU BOIS.

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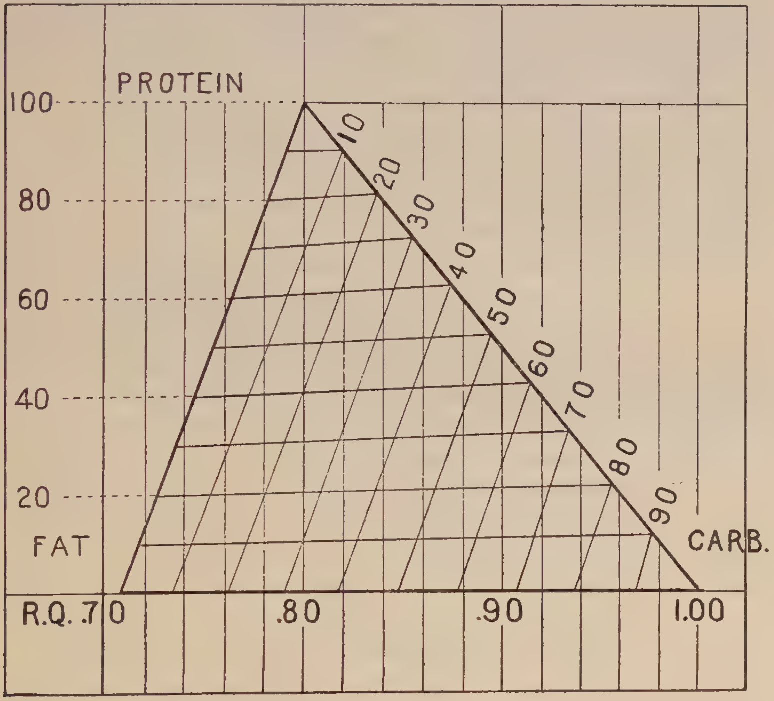
The percentages of calories furnished by the various foodstuffs can be shown graphically and with satisfactory accuracy on a chart which is almost an equilateral triangle. The base represents the respiratory quotient. The lower left hand corner

(R. Q. 0.707) represents the theoretical point at which 100 per cent of the calories are obtained from fat, the lower right corner (R. Q. 1.00) represents 100 per cent of the calories from carbohydrate. The top of the triangle, which is over the R.Q. of 0.801 is the protein corner. The percentage of calories furnished by protein is shown by means of lines almost parallel to the base. The percentage furnished by carbohydrate is shown by lines parallel to the left side of the triangle. These parallel lines are almost but not quite equidistant.



Almost all the phenomena of the respiratory metabolism can be represented graphically on this "metabolism map" and we can follow the changes which result from the ingestion of protein or carbohydrate. In the lower left hand corner is the zone of ketosis. Outside and to the left of the triangle is the zone in which protein is partially converted into carbohydrate. To the right of the triangle is the zone in which carbohydrate is converted into fat.

34 (2266)

The effect of suprarenalectomy in the rat on agglutinin formation.

By HENRY L. JAFFE and DAVID MARINE.

[*From the Division of Laboratories, Montefiore Hospital, New York City.*]

Many general facts have been accumulated from chemical and pathological studies which show that the suprarenal glands are in some way involved in the resistance of the organism to infections and intoxications.

Immunological studies in relation to suprarenalectomy are very limited. Gates, in 1918, was unable to demonstrate any noteworthy difference in hemolysin and agglutinin formation in a small series of partially suprarenalectomized guinea pigs and rabbits. Také and Marine have recently reported that rabbits with high grade but sublethal insufficiency showed hemolysin titers averaging more than twice as high as the controls.

The difference in results probably depends largely on the degree of suprarenal insufficiency produced. Removal of one suprarenal will rarely produce appreciable insufficiency, while removal of one gland and a portion of the other leaves behind tissue that possesses a great but variable and uncontrollable capacity to undergo regeneration. The most suitable laboratory animals for the study of suprarenal insufficiency are rabbits and rats in whom both main glands may be removed with a fairly high percentage of survival. Approximately 50 per cent of rabbits and 75-80 per cent of rats will survive this operation.

We wish to report the results of further studies in which rats were used as the test animal and agglutinin formation as the indicator of reaction. The rat compensates rather rapidly following removal of both main glands as has been shown by numerous studies on its susceptibility to a large variety of poisons. It is evident, therefore, that in studies involving suprarenal insufficiency in the rat, one must take into consideration the time factor in addition to removal of both main glands.

In a series of preliminary tests for agglutinin formation we used rats that had survived suprarenalectomy for from 30 to 50 days. Agglutinin titers for these animals showed no noteworthy differences from their strain, age and weight controls. Some of these rats received as high as 2 cc. of standard typhoid vaccine with no clinical reactions. The conclusion drawn from these experiments was that rats which had survived suprarenalectomy in excellent condition for from 30 to 50 days had compensated and were unsuitable for studies involving suprarenal insufficiency.

We, therefore, began to immunize animals 8 and 13 days after suprarenalectomy. No animals were used unless they were alert, active, eating, and had not lost appreciably in weight. It is the general experience that most rats in good condition one week after operation would probably make a complete recovery and survive indefinitely.

Four animals, two males and two females, that were suprarenalectomized eight days previously were injected together with four strain, age, sex, and weight controls with 0.25 cc. of vaccine. All the operated animals reacted severely to the injection, becoming dull, weak, and unable to walk; three died within six hours following the injection, while the controls showed no reactions. Normal rats of similar weight and age readily withstand 5 cc. of vaccine. This series demonstrated a marked diminution in the resistance of recently suprarenalectomized rats to typhoid vaccine. The surviving operated rat and two controls were given two additional doses of 0.25 cc. of vaccine on the third and sixth succeeding days and were bled from the tail on the third, sixth and ninth days following the last injection. The highest titer developed by the operated rat was 1200, while the highest developed by one normal rat was 200 and by the other 600.

As a result of the high mortality in immunizing rats eight days after suprarenalectomy, two additional series comprising nine suprarenalectomized and nine control rats were immunized beginning the thirteenth day after operation. All rats survived the 0.2 cc. vaccine given at three day intervals; the controls showed no reactions while most of the suprarenalectomized rats became dull and weak immediately after injection but recovered before the next injection. The animals were bled from the tail on the third, sixth and ninth days after the last injection.

The average titers of the suprarenalectomized animals for the first, second and third titrations were 900, 750 and 650, while those of the controls were 400, 250 and 250. In other words, the titers of the suprarenalectomized animals were between two and three times higher than those of the controls.

SUMMARY

These studies indicate that recently suprarenalectomized rats may succumb to one-twentieth of the dose of standard typhoid vaccine which normal rats readily withstand. Further, it has been shown that consideration of the time factor is important since animals tend to compensate for a suprarenal insufficiency by hypertrophy of accessory or unremoved portions of the main glands. Failure to take these features into consideration accounts in large part for the conflicting statements that have appeared in the literature. Finally, the fact that recently suprarenalectomized animals show a much higher antibody response to a given dose of antigen than do normal animals, clearly indicates that the suprarenal glands play a more important rôle in immunologic reactions than has heretofore been suspected, and it is our belief that this phenomenon is principally associated with the cortex or interrenal gland.

35 (2267)

The acid-base balance in pneumonia.

By A. B. HASTINGS, J. M. NEILL, H. J. MORGAN and C. A. L. BINGER.

[*From the Hospital of The Rockefeller Institute for Medical Research, New York City.*]

As a part of the study being made of the respiratory and metabolic disturbances occurring in pneumonia, the acid-base balance and the arterial oxygen unsaturation of pneumonia patients have been determined. Thirty observations have been made on 16 patients. The pH was determined directly by Cullen's colorimetric method and checked in 12 instances by electrometric measurements. The CO_2 content, O_2 content and O_2 capacity were determined on the Van Slyke constant volume blood gas apparatus. The analyses to be reported were performed on arterial blood drawn at least once from each patient during the height of the disease and in some instances in the post-febrile period.

The results when plotted on the Van Slyke acid-base diagram, so modified as to include iso-CO_2 tension lines, indicated that almost all of the points were well within the normal acid-base area. No instance of uncompensated acidosis was encountered. In three cases the points fell just outside the normal acid-base area in the compensated acidosis region. One of these patients was afebrile at the time and the other two were suffering from renal disorders. In no instance was there an acidosis of sufficient severity to indicate the advisability of bicarbonate therapy. It has been concluded from the observations thus far that the acid-base condition of patients suffering from pneumonia is usually within normal pH, CO_2 content and CO_2 tension limits.

In eight instances in which we have made repeated observations of the acid-base condition of the same patients we have found evidence that during the febrile period, the CO_2 tension has a tendency to be lower and the pH higher than after the temperature has returned to normal. These results indicate that during the febrile period there is over-stimulation of the respiratory mechanism which leads to a lower CO_2 tension and a slightly higher pH but that this disturbance does not usually remove the patient from a normal acid-base condition. No direct relation has been found between the degree of oxygen unsaturation and the low CO_2 tension of the febrile period.

36 (2268)

A study of the behavior of coal tar on the tissues.

By LOUIS H. JORSTAD (by invitation).

[*From the Department of Surgery, Washington University School of Medicine, and The Research Laboratories, Barnard Free Skin and Cancer Hospital, St. Louis, Mo.*]

In another study Burrows has shown that growth in a tissue depends on a crowding of the cells together and a cutting down of their relative blood supply. It is the direct result of the accumulation of a substance which stimulates the normal metabolism of the cell. This stimulating substance is soluble in the circulating body fluids. He failed to find any direct difference between the cancer cell and the normal cell. The independent growth seen in cancer like the shape and the arrangement of the cells may be directly related to a primary change in the normal orderly arrangement of the cells in the tissues of the body.

This growth stimulating substance is liberated in the normal metabolism of the cell. It acts directly to break down the proteins. In excess it causes a complete destruction of the cell. In less concentration it causes growth. Dense masses of cells poor in blood vessels or avenues of escape of this substance, such as cancerous tissues and the tissues of young embryos are rich in this substance. Other tissues cut off from their blood supply but supplied with oxygen accumulate this substance after a latent period which varies indirectly with the immediate cell density of the fragment of tissue being tested.

Cancer, as Burrows' studies indicate, is the result of any condition or substance which can cause the formation of a dense mass of cells relatively poor in blood vessels within the organism.

This stimulating substance as Burrows has further shown, acts to produce another substance in fixed tissue cells which is an active blood coagulant. This coagulating substance is poorly soluble in the fluids of the body but combines readily with fibrinogen to form fibrin. It flows readily over the surface of body fluids.

This coagulating substance again has strong affinities for the cell. All migration of the fixed tissue cells is the direct result of the absorption of this coagulating substance by substance or substances like fibrinogen, or the surfaces of the medium in the normal organism.

During migration this coagulating substance is taken away by the medium without. Fibrinogen becomes a stimulant for the migration of these cells in that it combines with this coagulating substance. It acts in this capacity only until it is completely saturated with this substance. In 1917 Burrows had already given evidence to show that this same substance decreases the surface tension of muscle cells in the interval between contractions and that the contractions are the result of an explosive breakdown of some substance increasing surface tension. In growing systems this same coagulating substance is formed in excess and disappears in the growing tissue. It is apparently combined or otherwise used in the formation of the protoplasm. Any actively growing tissues must drain the organism of substances leading to the formation of this coagulating substance as well as other important constituents for protoplasmic development.

Fischer several years ago had shown that drops of olive oil containing Scharlach R induce a rapid migration of epithelial cells to them. Recent and older careful studies of blood coagulation have shown that the coagulating substance of the body is probably a phospholipin. The action of these oily substances as well as coal tar may be none other, therefore, than the result of the absorption of this coagulating substance by them.

To prove this possibility tiny and large quantities of coal tar have been introduced just beneath the epidermis, into the subcutaneous tissue and into growing embryomata. When tiny drops of coal tar are introduced just beneath the epidermis of a rat the epithelial cells migrate along the needle tract to the drops that surround them like they invade the plasma clot. This migration does not continue indefinitely but only to the apparent saturation of the tar. Many of the cells which reach the coal tar early disintegrate. Later ones form a collar about the drop of tar. This collar gradually increases in thickness as more and more cells arrive.

No mitoses are seen in the first cells to arrive. Mitoses became evident only as the mass thickens. If more tar be added after the first drop ceases to be active this migration is resumed. The cells coming in contact with the fresh tar degenerate. Later ones do not. The mass thus thickens until an active autonomously growing tissue is established.

In the subcutaneous tissue of adults the coal tar causes a similar migration of connective tissue and endothelial cells to it, an extensive hyalinization of the fibrous tissue, and a degeneration of the few cells accumulating about it. In the more cellular mesenchyme of the embryonic tissues dense masses of connective tissue cells thus develop. Growth becomes evident in these masses only when they have reached a large size or when the cells have become densely packed together. These masses become larger and larger as more tar is added. They eventually became sarcomatous.

From these observations it became evident that the whole action of coal tar may be none other than the absorption of a certain blood coagulating substance which is a product of the cell's metabolism. It acts thus to accumulate cells and form an organization consistent with an autonomous growth of these cells.

It seemed evident therefore that coal tar itself must be able to produce all the symptoms of cancer. Such an active absorption of this coagulating substance must lead to an active drainage from the cells of a substance essential for the life of the organism.

In further proof for this deduction it has been found that these particles of coal tar do not remain localized in the tissue but they may migrate long distances through the tissues. From the subcutaneous tissue this migration is by way of the veins. The migrating tar metastasizes, so to speak, like a sarcomata. By this course it reaches first the lungs. Particles have also been found in distant organs.[1]

Very large doses or very frequently administered doses not only cause this migration but they also cause malaise, pronounced cachexia like that of cancer, and death of the animals.

The whole action of coal tar as we have seen it, therefore, is that of a simple absorbent of a certain coagulating substance formed by the tissue cells. As Burrows experiments have shown, the energy for activity in the body is derived from this coagulating substance. The coal tar stimulates growth in that it removes the cells from their more active blood supply and crowds them together. Whether the active removal of this coagulating substance from the cell tends also to accelerate the metabolism of the cell is yet to be solved.

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[1] Since this study has been completed Mertens has noted the migration of tar from the skin of rats to internal organs, *Z. Krebsforsche,* 1923, xx, 211.

37 (2269)

Cultures of plant cells.

By WILLIAM H. CHAMBERS (by invitation).

[*From the Research Laboratories of the Barnard Free Skin and Cancer Hospital, and the Department of Surgery, Washington University School of Medicine, St. Louis, Mo.*]

It has recently been shown by Robbins[1] that excised seedling root tips about 1 cm. in length, when planted in flasks of sterile liquid or agar media, grow to a limited extent to form organized root tissue with secondary and tertiary root branches. It is interesting to note that no one has observed a tendency for these plant cells to separate as animal cells separate from fragments placed in a nutrient medium. In a recent series of experiments it has been found that the individual plant cells will separate and migrate away from the fragments if the fragments are of a definite size and are cut from a definite part of the plant. This migration is in every way similar to that seen in animal cells.

Squash tissue was used for this study. Seedlings were grown aseptically and fragments of primary meristematic tissue were excised from the root tip for culture. They were planted in a drop about 1 mm. thick of a nutrient salt medium similar to Pfeffer's, with the addition of 0.04 per cent peptone, 2 per cent dextrose and 0.6 per cent agar, and kept in the incubator at 27°C. The reaction of the medium was pH 5.6. The size of the fragments varied in diameter from 0.3 mm. and 2.5 mm. From fragments of this tissue 0.4 to 1.0 mm. in diameter the cells reacted like the cells of higher animals. Pieces of tissue 1 mm. or greater in diameter, on the other hand, grew to form organized root tissue having a length often as much as 8 to 10 mm.

In animal tissue Burrows has shown that the rate of cell migration and growth is related to the size of the fragment, the original cell density of the fragment, and the original blood supply. Why the plant cells in fragments of a certain size migrate, while in larger ones they remain together and grow to form an organized tissue has not been determined.

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[1] Robbins, W. J., *Bot. Gaz.*, 1922, lxxiv, 59.

In the cultures of fragments between 0.4 mm. and 1 mm. the individual cells migrated over the surface of the medium in some cases to a distance of 1.7 mm. from the tissue block. In a few cases marked elongation of the fragment took place subsequent to the migration. Migration occurred for the most part over the upper and lower surfaces of the drop of agar medium. Only occasionally did the cells invade the solid medium. On the lower surface a viscid film was plainly visible diffusing from the fragment. The individual cells were evenly distributed through this film which held them immobile against shaking. After 24 hours some of the isolated cells were plasmolyzed, others have remained viable for more than 30 days.

The migration of the plant cells from the smaller fragments is similar to that of the cells of the higher animals. In the growth of the larger pieces to form parts of a plant the reaction of the plant tissue is unlike that of the higher animals but resembles the growth seen in pieces of tissue of some invertebrates. These pieces may regenerate parts or whole animals.

38 (2270)

A new method of using phenolsulfonephthalein for testing renal function.

By SANFORD M. ROSENTHAL* (by invitation)

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

Following the intravenous injection of phenolsulfonephthalein, its rate of disappearance from the blood was studied in normal animals and in those with experimentally produced renal pathology. A dosage of 0.5 mg. of dye per kilo of body weight was found most satisfactory. A striking difference in results was found to exist between normal dogs and normal rabbits. This is shown in the following table; the figures represent the concentration of dye in the blood serum at the stated time after injection, the percentages expressing that part of the total amount injected.

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* Fellow National Research Council.

Time	Normal Dog	Kidneys Removed	Normal Rabbit	Kidneys Removed
minutes	per cent	per cent	per cent	per cent
5	10	44	46	54
15	3	35	23	45
30	0-trace	28	13	36
60	0	24	4 to 5	33

It is thus seen that immediately after injection, the sulfonephthalein is quickly concentrated in the kidney of the dog while this does not occur in the rabbit. This suggests the possibility of normally existing physiological differences in the renal mechanism of these animals.

Tests were carried out upon dogs with complete urinary obstruction, produced by ligating both ureters. The first day after operation the concentrations reached in the serum by the sulfonephthalein were only slightly higher than normal, becoming successively higher in tests performed each following day up to the time of death, so that in urinary obstruction where excretion tests could not be made, a study of the sulfonephthalein in the blood serum gave an index as to the conditions of the kidneys.

39 (2271)

A new method of testing liver function with phenoltetrachlorphthalein. IV.

By SANFORD M. ROSENTHAL* (by invitation.)

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

A. FUNCTIONAL CHANGES AFTER REMOVAL OF LIVER TISSUE

The author's method of testing liver function, by determining the rate of removal of phenoltetrachlorphthalein from the blood stream was carried out in a series of 11 normal rabbits and repeated immediately after the removal of varying amounts of hepatic tissue. The relation between the degree of retention of tetrachlorphthalein in the blood and the quantity of liver substance removed was strikingly uniform. Slight deviations that fell within the range of normal variations first appeared when one tenth to one eighth of the liver was removed. The curve of disappearance of tetrachlorphthalein from the serum after 20 per cent of the liver was removed was 3.14 times as high as the average normal curve. Following removal of 81 per cent of the liver the curve was 7.37 times normal, and with the entire liver out it was 12.78 times higher than normal. (The surface area method of comparison of these curves, originated by P. D. Lamson, was employed.) These experiments establish the sensitiveness of the test and show that these results bear a quantitative relation to the amount of liver tissue in normal animals. In any application of these findings it must be borne in mind that these experiments dealt with normal liver tissues, and that the tests were carried out immediately after the removal of liver tissue.

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* Fellow National Research Council.

B. LIVER FUNCTION IN SYPHILIS AND IN ARSPHENAMINE JAUNDICE

Hepatic function tests with phenoltetrachlorphthalein were carried out in 22 cases of untreated syphilis in various stages, and in 11 cases of jaundice following arsphenamine therapy. In 14 cases of untreated tertiary syphilis with no manifestations except positive Wasserman reaction, liver function tests with tetrachlorphthalein were normal. In one case of primary syphilis a normal test was obtained. In 6 cases of secondary syphilis with skin eruptions, slight impairment of hepatic function was present in all.

Twenty-one tests were performed on 11 cases of arsphenamine jaundice. In 4 tests performed within 10 days after the onset of jaundice, 25 to 30 per cent of the dye was present in the serum one hour after injection, when normally it is practically absent. This degree of retention is amongst the highest that the author has so far obtained in any type of liver disease. Tests performed later than 2 weeks after the onset of jaundice showed a gradual return to normal or almost normal function within 3 to 4 months. This severe early functional impairment with gradual return to normal speaks in favor of a destructive process with repair rather than a simple obstructive lesion.

C. PERMANENT STANDARDS

Advantage has been taken of the "compensator" method of comparison introduced by Walpole for the colorimetric determination of hydrogen ion concentration. With the aid of Dr. Dunning of Hynson, Westcott and Dunning, the "compensator" principle has been applied to the present purpose, and I have found it satisfactory in the estimation of phenolsulfonephthalein and phenoltetrachlorphthalein in the blood serum, urine, feces, and duodenal contents, as it obviates the necessity of chemical extraction. To determine quantitatively the phthaleins, or any indicator dye, in the blood serum, the serum is placed into two small test tubes. One tube is allowed to remain clear, or if necessary clarified, by the addition of 3 per cent hydrochloric acid. This clear serum is placed in a compensator box in front of a tube of the aqueous standard of the dye, and by looking through both tubes simultaneously, they are compared with the tube of alkalinized (colored) serum, behind which may be placed a tube of clear water. Aqueous standards of phenoltetrachlorphthalein, of strengths previously described, may be sealed in hard glass ampules.[1] This procedure does away with the necessity of preparing fresh standards for each tetrachlorphthalein hepatic function test. It was independently employed for this purpose by G. O. Broun of Boston.

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[1] Standards for phenoltetrachlorphthalein and comparator box may be obtained from Hynson, Westcott and Dunning of Baltimore. Artificial color standard tubes prepared from commercial dyes to exactly match the color values of the phenoltetrachlorphthalein dilutions have recently been made by H., W. & D. The artificial colors have the advantage of withstanding exposure to light and are indefinitely stable.

40 (2272)

Application of author's precipitation test to spinal fluids.

By R. L. KAHN.

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

This paper will give a resumé of the author's test for syphilis as applied to serum[1] and an outline of the application of the test to spinal fluids.

I. ANTIGEN

1. *Preparation of Alcoholic Extract:* Beef heart muscle is ground, dried and powdered in a manner usually employed for antigen preparation.[2] About 50 gm. of powdered muscle are placed in a 500 cc. Erlenmeyer flask and about 250 cc. ether added and shaken. This is extracted over night at icebox temperature. The ether is filtered off and about 150 cc. fresh ether added to the powdered muscle. Extraction is further carried on over night in the icebox. After 3 or 4 ether extractions, the supernatant ether is practically free from coloring matter, when the extraction is completed. The ether is then filtered off and the powdered muscle is dried at room temperature until free from ether odor. The ether extracts are either discarded or the ether redistilled.

The dried material is weighed; placed in a 500 cc. Erlenmeyer flask, and 5 cc. of 95 per cent alcohol added per gram of material. This alcohol extraction is carried out for about 10 days at icebox temperature with daily shaking. After this period, the extract is filtered off and kept in the dark as stock solution.[3]

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[1] The following are more recent discussions on this text: Kahn, R. L., Rapid Precipitation Phase of the Kahn Test for Syphilis, *J. Am. Med. Assn.*, 1923, lxxxi, 88. Holmes, Janet A., The Kahn Precipitation Test for Syphilis, *J. Am. Med. Assn.*, 1923, lxxxi, 294; Strumia, M. M., A Study of Serum Flocculation Reactions in Syphilis with Special Reference to the Meinicke, Sachs-Georgi, Kahn and Vernes Reactions, *Arch. Dermatol. and Syphil.*, 1923, viii, 50; Yagle, E. M., and Kolmer, J. A., The Kahn Precipitation Reaction in Leprosy, *Arch. Dermatol. and Syphil.*, 1923, viii, 183; Detweiler, H. K., The Value of the Kahn Precipitation Test for Syphilis, *J. Am. Med. Assn.*, 1923, lxxxi, 815; The Kahn Precipitation Test in a Public Health Laboratory, *Public Health J.* (Canada), 1923, xiv, 464; Anderson, J. F., and Fisher, E. E., A Comparison of the Wassermann and Kahn Tests in Three Hundred and Twenty-nine Cases, *N. Y. Med. J.*, 1923, cxviii, 490; Fox, J. C., Jr., and Sanderson, E. S., Observations on the Kahn Precipitation Reaction for Syphilis, *Am. J. Syphil.*, 1923, vii, 687.

[2] Powdered beef heart is now obtainable on the market from the Digestive Ferments Company, Detroit, Mich.

2. *Cholesterinization of Alcoholic Extract:* A given amount of extract (enough for about a month's use) is measured into an Erlenmeyer flask and cholesterin added to it in the proportion of 6 mg. per cc. The cholesterin is dissolved by warming the flask in the water bath and rotating. The cholesterinized extract is then filtered to free it from impurities, when it is ready for use in the tests.

3. *Titration of Cholesterinized Extract:* This is to determine proper proportion of antigen and saline to mix for the tests. The smallest amount of saline which, added to antigen, produces a precipitate capable of going back into solution upon further addition of saline, represents the desired amount to mix with antigen for use in the tests.

Method: Five 1 cc. quantities of antigen are measured into small test tubes of about 5 cc. capacity. To five similar tubes are added 0.6, 0.75, 0.9, 1 and 1.2 cc. saline, respectively. The saline in each case is poured into the antigen tube and the mixture immediately poured back and forth several times. A heavy precipitate will be present in each of the tubes.

A preliminary test of the solubility of these precipitates is carried out as follows: A small quantity, such as 0.05 cc. of each of the precipitates is measured into five clean tubes and saline (from 0.3 to 0.5 cc.) is added to each of these tubes. The precipitates in the tubes containing 1 cc. quantities of antigen and 0.6 or 0.75 cc. of saline are usually insoluble in saline; the precipitate in the tube containing 0.9 cc. saline may appear questionable, whereas the precipitates in the remaining tubes are usually soluble in saline.

The final observation of the solubility of these precipitates in saline is made one-half hour after originally mixing antigen and saline. The antigen-saline proportion containing the smallest amount of saline which gives a precipitate still soluble at the end of this half-hour period is the proportion to be used in the test.

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[3] Rubber stoppers should be avoided in connection with antigen—and corks should preferably be covered with tin foil.

TYPICAL ANTIGEN TITRATION

Tubes	1	2	3	4	5
Antigen cc.	1	1	1	1	1
Saline cc.	0.6	0.75	0.9	1.0	1.2

The saline is poured into the antigen in each case and the resulting mixture immediately poured back and forth several times.

Tubes	1	2	3	4	5
Results	Pptate	Pptate	Pptate	Pptate	Pptate

The solubility of each precipitate is tested (immediately and a half hour after mixing saline with antigen) by placing a small amount, such as 0.05 cc., into a tube and adding about 0.5 cc. salt solution.

Tubes	1	2	3	4	5
Final Results after half-hour	Pptate	Pptate	Pptate?	No Pptate	No Pptate

According to this titration, tube No. 4 (1 cc. antigen + 1 cc. saline) contains the smallest amount of saline in proportion to antigen which will produce a precipitate capable of going back into solution in saline. This particular antigen, therefore, should be mixed for the tests in the proportion of 1 cc. + 1 cc. saline.

An important factor in mixing antigen with salt solution is that the latter should come in contact with the former with reasonable rapidity. This explains why the saline is added to the antigen by pouring it from another tube rather than from a pipette. When employing a small amount of antigen for the titration, such as 0.5 cc. or less, the saline should preferably be added from a 1 cc. pipette which must, however, permit a fair sized stream.

It is recommended that an antigen be re-titrated at periods of four to six weeks.

II. THE TEST WITH SERUM

1. *Dilution of Antigen for the Tests.* This should be carried out just before using in the test. The antigen-saline mixture will usually not keep longer than one-half hour.

(a) A given amount of cholesterinized antigen is measured into a small tube.

(b) An equal amount of saline is measured into another small tube (based on titration).

(c) The saline is poured into the antigen tube and the mixture is immediately poured back and forth several times. This mixture is now ready for use. (If 0.5 cc. or less of antigen is employed, the saline is added with a pipette.)

2. *The Test Proper.* The antigen-saline mixture and serum are added to three tubes in the proportion of 1:3, 1:6 and 1:12.

The following outline is based on quantities employed in this laboratory. The antigen mixture is always pipetted first and to the bottom of the tubes.

Tubes	1	2	3
Antigen-Saline Mixture cc.	.05	.025	.0125
Serum (heated 30 min. at 56° C.) cc.	.15	.15	.15

3. *Completion of Tests.*

(a) After adding serum to the antigen-saline mixtures, the racks are shaken vigorously for one minute.

(b) The tests are now ready to be recorded. To simplify reading, an amount of saline is added to each tube, sufficient to render the negatives so clear that they can be read without being removed from the racks. From 0.3 to 0.5 cc. saline will usually suffice. This should be added immediately before reading.

(c) The results should be read in front of a window with a darkened background. Each tube suggesting a positive reaction is lifted above the eye level, slanted to spread the fluid into a thin layer and examined for a precipitate.

(d) Readings are recorded on the basis of one, two, three and four plus, depending upon the distinctness of the precipitate. The final result is the average reading of the three tubes. Any given reaction will show quantitatively fewer particles in the tubes containing the smaller quantities of antigen, but, if the precipitation is complete in all three tubes, it should be read four plus in spite of the quantitative difference in the number of particles.

(e) A check reading of the tests should be made, preferably by another worker. If it is necessary for the same worker to make this reading, a better check will probably be obtained if made at a later period. In such a case, the racks should be kept in the icebox until the check reading is made.

Note: Studies on the effect of incubation on this test indicate that, if carried out after the shaking period and before the addition of saline, 15 minutes to an hour (or even longer) at 37° C. does not produce falsely positive reactions.

4. *Controls.*

(a) Antigen Controls: After mixing antigen with saline for the tests, a small amount, such as 0.05 cc., of the mixture is measured into a tube and 0.5 to 1 cc. of saline added. There should be no sign of precipitation. Also, when pipetting antigen for a series of tests, the last set-up is used with saline instead of serum and is read with the regular tests.

(b) Serum Controls: All serums should be observed for precipitates prior to their use in the tests.

(c) Positive and Negative Controls: Three positive and three negative controls should be used with each set of tests.

III. THE TEST WITH SPINAL FLUID

Although this precipitation test gives good results with strongly reacting spinal fluids when used in the same proportion as serum, the test lacks sensitiveness when used with moderately and weakly positive fluids. It was believed that better results could be obtained by precipitating and subsequently concentrating the proteins—and therefore the reacting substances—of the fluid. Various methods of protein precipitation are still under investigation in this laboratory. Recently, Herrold[4] suggested precipitating the globulins of spinal fluid with ammonium sulfate in connection with his ring modification of the writer's test.[5] This ammonium sulfate precipitation method, when applied to our test, has given good results. The procedure to be outlined involves: First, precipitating the globulins in the spinal fluid with ammonium sulfate and concentrating this precipitate in saline; second, testing this concentrated solution for the presence of syphilitic reacting substances by means of the regular precipitation test.

Procedure: The spinal fluid is mixed with saturated ammonium sulfate (C. P.) to produce 40 per cent saturation. Thus, 3 cc. of fluid are mixed with 2 cc. ammonium sulfate; 4.5 cc. fluid with 3 cc., etc. This mixture is allowed to stand for about an hour at room temperature. It is then centrifuged at high speed for 10 minutes and the supernatant fluid poured off as completely as possible. The last drop adhering to the lip of the tube can be removed by means of filter paper. The resulting precipitate will be found suspended in a small drop of fluid. This precipitate is then dissolved in an amount of saline equal to one-tenth the volume of spinal fluid used in the test. (A precipitate from 3 cc. of fluid, for example, is dissolved in 0.3 cc. saline.) The saline should be added to the bottom of the tube to prevent washing down traces of ammonium sulfate from the inner wall. The globulin solution formed is now ready to be tested for specific precipitation with the antigen-saline mixture described above.

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[4] Herrold, R. D., The Precipitation Test for Syphilis of Concentrated Arachnoid Fluid and Serum, *J. Am. Med. Assn.*, 1923, lxxxi 203.

[5] Herrold, R. D., A Ring or Contact Precipitation Test for Syphilis, A Modification of the Kahn Test, *J. Am. Med. Assn.*, 1923, lxxix, 957.

In carrying out the test, two tubes are employed with different proportions of antigen and concentrated fluid. Tube 1 receives 0.01 cc. antigen-saline mixture followed by 0.1 cc. of concentrated spinal fluid. Tube 2 receives 0.005 cc. antigen mixture with also 0.1 cc. of fluid. The antigen should be measured with a 0.1 cc. pipette graduated in 0.01 cc. and the respective amounts deposited at the bottom of the tubes.

After adding the fluid to the antigen, the racks are vigorously shaken for 1 minute. To simplify reading, each tube receives 0.2 cc. saline, and the tests are read and recorded. The final result is the average reading of the two tubes.

The controls are: (1) Antigen control, same as with serum test. (2) Concentrated fluid, to be observed for the presence of a precipitate prior to its use in the test. (3) In view of the fact that an ammonium sulfate solution of sufficient concentration is capable of producing a precipitate with antigen, the following special control should be added to each series of tests. To 3 cc. saline are added 2 cc. ammonium sulfate to make a 40 per cent saturation. This is mixed and poured off in the same manner as the supernatant fluid of the spinal fluid-ammonium sulfate mixture is poured off after centrifugation. To the minute amount remaining in the tube is added 0.3 cc. saline and the resulting solution tested with antigen in the same proportions as the concentrated spinal fluid. (4) Positive and negative controls should accompany each series of tests.

This method compares favorably with the Wassermann test. Clinical observations with reference to specificity and sensitiveness will be reported in another place.

41 (2273)

The rate of growth of virulent and avirulent streptococci in serum media.

By FRANKLIN A. STEVENS.

[*From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*]

In a series of experiments which we reported in 1921[1] we found that after strains of beta type streptococci had been rendered virulent for mice by animal passage cultures of the virulent streptococci grew more slowly during the first few hours after they were inoculated into broth to which horse serum had been added than did cultures of the avirulent forms of the same strains. In comparing the growth of the virulent and the stock cultures of these streptococci bacterial counts were made at hourly intervals and the amount of hemolysin produced in the cultures was titrated with standardized suspensions of erythrocytes obtained from defibrinated horse blood. The amount of hemolysin produced at these intervals in these cultures corresponded with the rate of growth of the bacteria. The virulent streptococci grew slowly after they were inoculated into the broth, then after a latent growth period of several hours grew very rapidly until the sixteenth hour, after which the bacterial count gradually fell. The maximum amount of hemolysin was produced during the period of very rapid growth. The avirulent cultures showed a much shorter latent growth period. They began to grow rapidly after they had been inoculated into the broth about three hours and reached the maximum bacterial count from one to two hours before the cultures of the virulent bacteria had attained their maximum. The maximum amount of hemolysin was produced from one to two hours earlier in these cultures than in the cultures of the virulent streptococci although the maximum titers of hemolysin were equal. The avirulent forms of the streptococci always grew more rapidly under the conditions laid out for these experiments and the curve of the hemolysin titers was a constant index of the length of the latent growth period.

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[1] Stevens, F. A., *J. Exp. Med.*, 1921, xxxiii, 223.

In these experiments there were several factors which may have been responsible for these differences in growth. It was possible that the species of animal from which the serum in which the bacteria were grown was responsible. To eliminate this possibility, virulent and avirulent cultures of the same strain of streptococci have been compared in the serum of the animal for which they were rendered virulent.

Five strains of streptococci were inoculated consecutively into rats until .0001 cc. of 18 hour broth cultures killed these animals on repeated tests. Following out the technique of the previous experiments we have compared the growth and the production of hemolysin with these strains in broth enriched with rat serum. The hemolysin produced was titrated with standardized suspensions of cells obtained by defibrinating rat blood. The same results were obtained in these experiments as were found in those previously reported. The avirulent strains grew more rapidly immediately after inoculation and produced the maximum amount of homolysin earlier than the strains which had been rendered virulent for mice.

Since similar results were obtained in both series of experiments it appears that the differences in the rates of growth depend on the differences in the virulence of the strains. The experiments suggest that an analogy exists between the behavior of these strains of streptococci and saprophytic and parasitic bacteria. Saprophytes which grow readily *in vitro* resemble the less virulent cultures of streptococci, while parasitic, pathogenic bacteria which are grown with greater difficulty in media resemble the more slowly growing virulent cultures of streptococci.

42 (2274)

Studies in narcosis.

IV. A simple and rapid method for the determination of ether in blood.

By T. K. KRUSE.

[*From the Department of Physiology and Pharmacology, University of Pittsburgh, Pittsburgh, Pa.*]

The principle of the method here described depends upon the separation of ether from blood by boiling a tannic acid blood mixture and determining the amount of recovered ether by differential absorption in concentrated sulfuric acid. Obtaining the gas sample requires 5 minutes and the entire procedure, 15 minutes. The methods described in the literature are time consuming.[1, 2, 3]

A 30 cc. test tube is arranged in aspirator fashion. The rubber stopper is protected by a copper guard to reduce the exposure of rubber to ether vapor. Fifteen cc. of a 1 per cent tannic acid solution are put into the test tube to which 1 cc. of oxalated blood is added. The test tube is stoppered, the tube extending into the liquid is closed by means of a pinchcock and the tube extending only through the stopper is connected with a sampling tube containing concentrated calcium chloride solution covered with a generous layer of glycerol in which ether is practically insoluble. With the sampling tube under negative pressure, the blood mixture is boiled for a minute to expel all the gases dissolved in the liquid. Near the boiling point of the liquid the precipitated blood congeals and adheres to the walls of the test tube and does not interfere in any manner with the procedure. The ether which remains in the air of the test tube is very rapidly drawn into the sampler by opening the tube which extends into the liquid. It is important that this is done as rapidly as the apparatus permits to prevent the diffusion of gases. The 40 cc. sample thus obtained is transferred to the 40 cc. analyzer and the ether content determined.[4] The test tube may be readily cleaned of its sticky coagulum by washing with dilute sodium hydrate solution.

Weighed samples of unpurified ether when added to blood yielded 90 per cent or better of the theoretical value. Inasmuch as such ether is only 90 to 95 per cent pure the method is sufficiently accurate for pharmacological investigation. Blood containing no ether yielded no absorbable gas. The error of reading was 0.02 cc. but quite frequently identical readings were obtained on duplicate blood samples.

The concentrations of ether found in the blood are in harmony with those published in the literature, Table I.

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[1] Nicloux, *Compt. rend. Soc. biol.*, 1906, lxi, 606.

[2] Haggard, H. W., *J. Biol. Chem.*, 1923, lv, 131.

[3] Shaffer, P. A., and Ronzoni, E., *J. Biol. Chem.*, 1923, lvii, 741.

[4] Kruse, T. K., *J. Biol. Chem.*, 1923, lvi, 127.

TABLE I.

Protocol of Dogs Showing the Nature of Results Obtained.

Time A. M.	Temp. °C.	Ether in 100 cc.			Remarks.
		Arterial Blood		Air	
		cc.	mg.	cc.	
Dog A.					
10:45	39	----	------	------	Dog anesthetized.
11:02	39.1	41	136	6.63	Very slight corneal reflex.
11:18	39.3	32	106	4.50	Corneal reflex.
11:24	----	----	------	------	Ether concentration increased.
11:35	39.6	38	126	7.63	Arterial blood dark. No corneal reflex.
11:46	39.8	36	119	6.06	Arterial blood dark. No corneal reflex.
11:53	----	----	------	------	Ether concentration increased.
12:00 M.	39.7	43	142	8.67	Respiration shallow. Arterial blood dark. No corneal reflex.
Dog B.					
A. M.					
10:00	39.0	----	------	------	Dog anesthetized.
10:48	39.2	----	------	5.11	Corneal reflex.
10:56	39.2	----	------	4.31	Corneal reflex.
11:04	39.4	28	93	3.40	Corneal reflex.
11:12	----	----	------	------	Ether concentration increased.
11:18	39.5	32	106	5.70	Corneal reflex.
11:26	39.6	31	103	4.35	Corneal reflex.
11:37	39.6	31	103	4.57	Corneal reflex.
11:52	----	----	------	------	Ether concentration increased.
11:57	39.8	42	139	6.92	Quieter resp. Slight corneal reflex.
P. M.					
12:06	39.9	32	106	6.55	Slight corneal reflex. Arterial blood dark.
12:18	40.0	31	103	5.38	Corneal reflex.

43 (2275)

The absorption of digitalis from the rectum in man.

By ROBERT L. LEVY.

[*From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*]

Not infrequently, patients suffering from heart failure are unable to take digitalis by mouth because of nausea, vomiting or surgical operation. The margin of safety between therapeutic and toxic dose, when a member of the digitalis group is given by vein, is sufficiently small to render this method of administration hazardous. The present study was undertaken to ascertain the feasibility of rectal digitalis therapy, concerning which only a few fragmentary reports have been recorded.

Twenty-six observations were made on 20 patients with auricular fibrillation. Hourly records of heart and pulse rates were charted for 8 hours after the drug was given and at 4 hour intervals for the next 24 hours. In 19 instances, an electrocardiogram was taken just before administration and approximately every hour thereafter for 3, 4, or 5 hours. Another graphic record was made the following morning.

The preparation employed was an aqueous extract of digitalis leaves furnished by Merck and Company and called by them "Digitan." One cubic centimeter of the liquid contained the equivalent of 0.1 gm. of powdered leaf. Biologic assay was done by the 1 hour frog method.

The patient received a preliminary cleansing enema. After evacuation, 8 to 20 cc. of digitan were given by rectal tube and washed through with 25 cc. of water. The micro-enema was retained. Alcoholic extracts of digitalis were found to be irritating to the rectal mucosa unless diluted to such volume that, because of bulk, they were often expelled.

Retardation in ventricular rate occurred in every instance following digitalis administration. The average time necessary for an unmistakable initial effect on rate was 2 hours and 35 minutes. The interval ranged from 1 hour and 15 minutes to 7 hours and 40 minutes. The average time which elapsed before a maximal effect on rate was apparent was 9 hours and 30 minutes. This time ranged from 3 hours and 15 minutes to 22 hours.

A characteristic change in the T-wave of the electro-cardiogram occurred in 14 of the 19 observations. In 5 cases, no change was observed. The initial effect was seen, on an average, in 2 hours and 30 minutes; this was approximately the time necessary for a beginning fall in rate. This period varied in length from 1 hour and 20 minutes to 5 hours and 15 minutes. Usually, a greater change in the T-wave was seen in the curves made the following morning.

A desirable therapeutic effect was apparent in every case. In many of the patients, the results were dramatically rapid and beneficial. On two occasions there was nausea and vomiting. There were no other evidences of untoward digitalis action. A detailed report of the clinical aspects of this study will shortly be made.

44 (2276)

Experimental production of rickets with diets of purified food substances.

By THOMAS B. OSBORNE, LAFAYETTE B. MENDEL and
EDWARDS A. PARK.

[*From the Laboratory of the Connecticut Agricultural Experiment Station, the Sheffield Laboratory of Physiological Chemistry and the Department of Pediatrics, Yale University, New Haven, Conn.*]

The possibility of producing experimentally in animals symptoms essentially identical with those associated with rickets in human beings promises to advance the study of this disease greatly. The efforts in this direction have hitherto been concerned with the effects of diets of varied composition—usually for the most part mixtures of natural foods or materials derived therefrom without much manipulation. This has made comparisons between different rations somewhat difficult and often unconvincing because with a change in the natural foods several chemical ingredients are altered at the same time and consequently the cause of any marked change induced thereby in the animal usually cannot be charged directly to changes in any one chemical factor. For example, Sherman and Pappenheimer have demonstrated that rickets is brought about in a few weeks in rats by a diet of patent flour 95 per cent plus a mixture of three inorganic salts (Ca lactate 2.9 per cent, NaCl 2.0 per cent, Fe citrate 0.1 per cent). In experimental feeding tests under otherwise comparable conditions we found that the introduction of 10 per cent of a protein (lactalbumin was used) to replace an equal weight of flour in the Sherman-Pappenheimer ration increased the severity of the symptoms. The calorie value of the two foods was essentially alike. One diet was made far richer than the other in protein of good biological quality; but this result was attended with "dilution" of the flour so that the various food factors which it specifically introduced into the diet, viz., phosphates and certain other inorganic ingredients, vitamines, and carbohydrates, were decreased. Were the gains or the losses responsible for the increased severity of the rickets? Obviously the physiological "analysis" or interpretation becomes complicated where several food factors are altered by an even seemingly simple change in the diet.

This criticism applies to most of the investigations conducted hitherto on rickets from a dietary standpoint. It has therefore been difficult to point unmistakably to a single responsible feature in the midst of numerous simultaneously shifting variables. McCollum, Simmonds, Becker, and Shipley[1] realized this when they attempted to produce rickets in rats by the use of more refined food materials. Their diets still included such products as wheat germ, wheat gluten and agar-agar; and the animals "developed xerophthalmia notwithstanding the presence of sufficient fat-soluble A to cover the minimum nutritive needs of the rat."

In order to supply a diet in which, as nearly as the present state of our knowledge and the development of our technic will permit, one factor at a time can be modified, we have resorted to feeding tests with purified food materials and a salt mixture in which each element could be varied independently.[2] Although even this procedure does not entirely avoid all of the difficulties referred to, in so far as withdrawal or addition of one factor may alter the relative proportions though not the kinds of the remaining factors, it affects them far less than when natural products are used. This applies especially to the inorganic ingredients. In general the food mixtures have consisted of some or all of the following components: purified protein, starch, lard, salt mixture, Osborne-Wakeman yeast fraction in small quantity for vitamine B.

The "complete" salt mixture is composed of:

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[1] McCollum, E. V., Simonds, N., and Becker, J. E., and Shipley, P. G., *J. Biol. Chem.*, 1922, liv, 249.

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxiv, 121.

	gm.
$CaCO_3$	134.8
$MgCO_3$	24.2
Na_2CO_3	34.2
K_2CO_3	141.3
H_3PO_4	103.2
HCl	53.4
H_2SO_4	9.2
Citric acid + H_2O	111.1
Fe citrate 1 1/2 H_2O	6.34
KI	0.020
$MnSO_4$	0.079
NaF	0.248
$K_2Al_2(SO_4)_4$	0.0245

A comparable salt mixture can be prepared without calcium, magnesium, or phosphorus as may be desired. For example, the phosphorus-free mixture used was prepared from

	gm.
$CaCO_3$	122.4
$MgCO_3$	12.1
Na_2CO_3	17.1
K_2CO_3	35.3
HCl	53.4
H_2SO_4	9.2
Citric acid H_2O	95.0
Fe citrate 1 1/2 H_2O	6.34
KI	0.020
$MnSO_4$	0.079
NaF	0.248
$K_2Al_2(SO_4)_4$	0.0245

Young albino rats weighing about 50 grams were placed on selected diets for a period of 35 days whereupon they were killed and examined. When the food contains *all* the known essentials including protein of good quality, as indicated above, the male animals grow within this period to about 125 grams in body weight. Without vitamine A, growth is retarded; but histological examination of the bones fails to reveal any changes characteristic of rickets.

Marked rickets has appeared on diets consisting of

Protein (edestin or lactalbumin)	16-20 per cent	With a daily addition of 40 mg. yeast fraction.
Starch	56-52 per cent	
Lard	0-24 per cent	
P-free salt mixture	4 per cent	

The skeletons of the rats showed the gross and microscopic changes characteristic of rickets. The costo-chondral junctions were greatly enlarged and deformed. The lower ends of the radius and ulna were much enlarged. The femur cut easily. Microscopic examinations taken through its lower end disclosed the characteristic rachitic metaphysis. The cartilage was broadened, had grown in an irregular manner toward the shaft, and on the shaft side showed the finer metaplastic or degenerative changes found in human rickets. Calcification was either deficient or entirely lacking. The trabeculæ showed well-marked osteoid borders.

The foremost significance of these experiments seems to us to lie in the possibility that they present of modifying, independently, a number of factors that may influence the abnormal metabolism of the bones. The rôle of ions other than phosphate can be tested. The debated importance of carbohydrate in the genesis of rickets, the comparative importance of light versus the organic and inorganic factors, the calorie intake, the possible contributory significance of the protein element—these and other influences can perhaps better be evaluated experimentally in this way. We are engaged in the applications of the method to the problems suggested.

45 (2277)

On the feeding habits of oysters.

By THURLOW C. NELSON.

[*From the Department of Zoology, Rutgers College, New Brunswick, N. J.*]

Studies of the feeding habits of oysters have been in progress since the spring of 1919 to determine: (1) the duration of active filtration of water, and (2) the effects of salinity, temperature, light, food content, and turbidity of the water upon the rate and the extent of feeding. The animals were studied while lying on an oyster reef in their natural environment. By means of recording apparatus continuous kymograph records of all shell movements of the oysters for over 2 months were obtained. Water samples were taken at regular intervals of time and tide, and in addition whenever the animals closed completely or opened after a period of closure in excess of 2 minutes.[1]

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[1] Preliminary reports of the writer in *Anat. Rec.*, 1921, xx, 181; *Reports of the Department of Biology, N. J. Experiment Station* for 1920, p. 333, and for 1921, p. 293.

The investigations have been carried on in several different environments, under widely varying conditions, and warrant the following conclusions:

1. With the water temperatures of late spring, summer, and early fall oysters are active throughout most of the day and part of the night. At temperatures above 20° C. New Jersey natural oysters are actively passing water through the gills for an average of over 20 hours out of the 24.

2. A sudden drop in temperature may result in temporary decrease in the daily number of hours of activity.

3. Between 4°-6° C. there is a sharp decrease in the ciliary activity of oysters. Normal feeding occurs above this range, while almost no food is taken when the temperature of the water is below 4° C. Round[2] found active elimination from oysters at 9° C. but no reduction in the number of bacteria at 5° C. until after a period of 5 days.

4. The rate of filtration of water during any given period of time, as deduced from the rapidity and the extent of ejections of accumulated sediment from the mantle cavity, may vary widely independently of the temperature and the turbidity of the water.

5. Oysters do not necessarily feed at all times when water containing food particles is passing over the gills. Relatively little food is taken on the ebb tide and during the latter part of the night and the early morning.

From 60-70 per cent of the hours of inactivity (closure) of the oyster occur during darkness.

7. For oysters which have been living in water of fairly constant saline content there is a definite minimum salinity below which the bivalves become inactive. This minimum salinity is lower for oysters grown in brackish water than for those matured in waters of higher salt content.

8. No correlation could be shown between the food content of the water and the periods of inactivity of the oyster.

9. The effects of turbidity were observed under natural conditions and when the water was made to carry varying loads of silt up to the maximum. Oysters continued to feed in waters of maximum turbidity (T = 1000+), the effect of the heightened load of silt being evident in the increased rate and depth of contraction of the adductor muscle to effect ejection of accumulated mud.

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[2] Round, L. A., Contributions to the bacteriology of the oyster. 1914—*Report, R. I. Com. Shellfisheries,* Providence.

46 (2278)

Studies on the pathological effects of organisms of the enteritidis paratyphoid B. group on the pancreas, liver and kidneys.

By BERNARD G. H. THOMAS (by invitation).

[*From the Pathological Laboratories, University of Pittsburgh, Pittsburgh, Pa.*]

During the fall of 1922, in an attempt to produce a streptococcic glomerulo-nephritis it was observed that pathological changes similar in character occurred in both the treated and the control animals. About the same time many of the stock guinea pigs began to die spontaneously and post mortem cultures of their organs and heart's blood yielded Streptococci, Staphylococci and organisms of the Enteritidis-Paratyphoid B. group, but the former were thought to be secondary invaders in a terminal septicæmia. The latter bacilli consisted of two distinct forms namely, *B. enteritidis* and *B. paratyphosus* of animal origin, separable by direct agglutination. Microscopic examination of the organs of the animals dying spontaneously showed that characteristic lesions were present in the kidneys, liver and pancreas. On injection of either killed or living bacilli of the Enteritidis-Paratyphoid B. group similar lesions, though more intense and widespread, were likewise obtained.

In the kidneys the most prominent changes occurred in the glomerulus, where considerable intra- and extra-glomerular hemorrhage, thrombosis and varying degrees of necrosis and disintegration were observed. Occasionally hyperplasia of the endothelial lining of the glomerular vessels was present. A marked degree of parenchymatous degeneration occurred primarily in the proximal convoluted tubules and to a less degree in the distal convoluted tubules and the ascending limb of the loop of Henle. In the very acute cases the congestion and hemorrhage had become extreme. Rarely small areas of round celled infiltration occurred.

The liver was characterized by marked congestion, focal necrosis and parenchymatous degeneration. The areas of focal necrosis varied considerably in size, the larger ones having been converted into typical abscesses with thin enveloping walls of fibrous connective tissue. These consisted of varying amounts of necrotic tissue, infiltrated with polymorphonuclear leucocytes mainly, although occasionally endothelial cells were numerous. The liver cells were pale, swollen, and showed considerable fatty degeneration and pigmentation.

The pathological changes noted in the pancreas were confined chiefly to the islets of Langerhans. These were characterized by an extreme degeneration which caused them to appear as exceedingly pale translucent patches clearly demarcated under low magnification from the acinar tissue. Even small islets consisting of one or two cells could be readily distinguished at a glance. Two types of cellular degeneration were found within the islets, one hydropic in character, the other pycnotic. The cells showing the hydropic degeneration were enormously swollen, the cytoplasm was pale and consisted of a wide meshed finely granular net work enclosing vacuoles of different sizes. In many of the cells the vacuolation had become so extreme that the thread work looked almost invisible and the cell appeared to consist of large watery vesicles. Frequently these cells had become ruptured leaving large pale nuclei with shreds of adherent cytoplasm and other debris. In the second type of degeneration the cells were not much altered in size and the nucleus appeared shrunken and pycnotic, and stained intensely. Both types occurred in proportions varying with different animals. We were not able, by means of the technique of Lane and of Bensley for the identification of the alpha and beta granules, to determine definitely of what type of cell the degenerated islet tissue was composed. Since in some of the cells showing the earliest stages of degeneration granules of the beta type could be faintly recognized it was thought that the larger part of tissue affected belonged to this type of cell. In the markedly degenerated islet tissue no granule stain whatever was obtained. The hydropic degeneration observed by us, in so far as can be determined, is identical with the morphological alterations in the islets produced by Homans and by Allen through a depletion of the islet cells following partial pancreatectomy and subsequent overstimulation of the remaining pancreatic tissue by feeding a high carbohydrate diet.

Experiments now being carried on in our laboratories and shortly to be reported indicate that a correlation exists between the pathological changes in the islet cells and variations in the sugar content of the blood.

47 (2279)

Studies on cancer.
I. The effect of circulation on the functional activity, migration and growth of tissue cells.

By MONTROSE T. BURROWS.

[*From the Department of Surgery, Washington University School of Medicine, and the Research Laboratories of the Barnard Free Skin and Cancer Hospital, St. Louis, Mo.*]

In a previous article I had shown that rhythmical muscular contraction as it occurs in fragments or the whole hearts of embryos in simple hanging drop cultures is regular only for a few hours. After this it becomes irregular; there are periods of activity followed by periods of rest. These active periods are ushered in by rapid strong contractions which gradually become less and less forcible and rapid until they finally cease. After a shorter or longer rest period of several minutes, the contractions begin again, as before.

These irregularities I had long ago concluded were due to the accumulation of waste products about the contracting cells. In the simple hanging drop cultures the only means of escape of such substances is by diffusion into the outer portion of the hanging drop of medium. I prepared at that time a culture[1] which allows one to wash continuously a greater part of such hanging drops of plasma with a stream of serum. The serum in this apparatus is dropped at the rate desired on the upper end of a wick which passes to the hanging drop and then into a receiving chamber. In the hanging drop on the surface of a cover glass the fibres of the wick are teased apart so that they transverse all parts of the hanging drop. The fragment of tissue or cells to be tested is placed in the center of the hanging drop. Plasma is used as the medium for the hanging drop. It is allowed to clot before the stream of serum is started.

In such cultures I found that as the serum is passing the contractions of the heart become regular and forcible in those cells which are sufficiently separated to be well washed by the serum. In the more compact part of the fragments the contractions are irregular as before. Again it is interesting to note that these rhythmical contractions of the muscle cells can be prolonged by this method. One group of cells in one of the fragments continued to contract for as long as 28 days. In the hanging drop cultures all activity ceases after 8 or 9 days as a rule. A few have contracted, however, at intervals up to the 18th day.

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[1] Burrows, M. T., *Anat. Rec.*, 1912, vi, 141.

In a later study I investigated the migration of the embryonic heart muscle, connective tissue and epithelial cells by the same method. About carefully cut compact fragments the migration of the cells is little disturbed over that seen in the simple hanging drop cultures. The migration begins after a given latent period, continues active for a time and then ceases. The area covered by new cells is the same as if the serum had not been passed through the culture medium. This had led me to believe in my earlier studies that the passing serum had no effect on, or possibly accelerated the migration of these cells.[1 2]

About the fragments which were teased apart, however, this is not true. I had noted at an earlier time that teasing itself always leads to a marked decrease in the extent of migration. Washing further decreases the migration of the mesenchyme, muscle and connective tissue cells. In the more compact epithelial membranes, however, this effect of washing is less marked. Breaking up these epithelial membranes lessens the movement. The single cells will not migrate. Washing small groups of these cells has little further effect upon this activity.

What is true for the fixed tissues is not true, however, for the lymphoid cells migrating from the spleen. The migration of these cells is decidedly affected by the flowing serum. In several instances the entire fragment of spleen disappears. All the cells migrate out as lymphoid cells. They accumulated at tangle portions of the wick. In the simple hanging drop culture such a complete dissipation of the cells never occurs from these fragments. When the fragments are transplanted repeatedly, a few may so dissipate. Many of the fragments of spleen cease, however, after a few transplants to new plasma to give forth lymphoid cell; connective tissue-like cells migrate from them. In many of the cultures which were washed with serum all the cells of the spleen became transformed into lymphoid cells and migrated out.[2] In a few of the cultures washed with serum I noticed also connective tissue-like cells migrating later from these splenic fragments. Whether this complete transformation of all the cells of the fragment into lymphoid cells in one case and as part lymphoid and part connective tissue-like cells in others is dependent alone on the serum is yet to be established. In all of these cultures, as it must be noted here, however, the migration of the wandering type of cell from the spleen is increased by the flowing serum. This fact places these cells in definite contrast to the fixed tissue cells.

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[2] Burrows, M. T., to appear in *Sci. Bull., U. of Kans.*, 1923.

Having established these facts it became of interest to study the effect of this flowing serum upon the growth and division of embryonic heart muscle cells and mesenchyme cells in these cultures. The lymphoid cells have never been seen to grow and divide in these cultures. The cells from fragments of young embryonic heart muscle fragment and fragments of body wall of chick embryos migrate with great activity after a short latent period, grow and divide by mitosis and later degenerate. The flowing serum not only delays slightly the migration of the cells from the compact fragment but inhibits decidedly the growth and division and delays greatly the degeneration of the cells in the outer medium. How long it may delay this degeneration has not been determined. The complications of preparing and handling the serum leads generally to infection sooner or later. Stopping the flow of serum allowed growth and the degenerative changes to proceed again.

While it is impossible by such a device to duplicate completely the circulation as it exists in the body there seemed little doubt that, quite the reverse of function, the growth and the migration of fixed tissue cells is inhibited by an active circulation. Growth fails in all parts of these cultures where the cells are well washed by the serum.

48 (2280)

Studies on cancer.
II. The significance of the effect of circulation on the growth of cells.

By MONTROSE T. BURROWS.

[*From the Department of Surgery, Washington University School of Medicine, and the Research Laboratories of the Barnard Free Skin and Cancer Hospital, St. Louis, Mo.*]

As early as 1913[1, 2] I had already shown that the growth and migration of fixed tissue cells is proportional at all times to the size of the fragment, the cell density of the fragment and indirectly proportional to the thickness of the layer of the medium. All of these proportions hold, however, only to the limits of an active oxygen diffusion to all parts of the culture. Oxygen, as I have pointed out, diffuses readily only 0.5 to 0.7 mm. into clots and later I have found that it diffuses readily no greater than 1 mm. into many dense fragments of tissue. For any of these fixed tissues the maximum activity of the cell is seen about fragments 1 mm. in diameter planted in layers of medium 0.5 mm. in thickness.

Single cells cannot grow in the plasmatic medium of the culture. Connective tissue and mesenchyme cells may stretch out to take their characteristic shapes and migrate short distances. These cells will not grow. Active migration is always proportional to the size of the fragment up to the limits cited above. In the primary plasma cultures an active growth of the mesenchyme cells is seen only about the cellular fragments of the tissues of young embryos and malignant tumors. The cells from these fragments suffer not only this active growth reaction but they later suffer frequently a complete degeneration in the cultures.

This degeneration as it is seen in these active growing cells is peculiar to all of the cultures. It commences early in the centers of all fragments except the least cellular ones cut from scars and adult subcutaneous tissue. It spreads outwards from this center. In the cultures of the more cellular tissues it involves sooner or

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[1] Burrows, M. T., *Trans. Cong. Am. Phys. and Surgeons*, 1913, ix, 77.

later all the cells except those which migrate directly into the plasmatic medium and out of the line of diffusion of substances from the fragment along the surface of the medium. About the less cellular fragments it never involves the cells which have migrated into the medium. These cells come to rest after a short time unless the medium undergoes liquefaction as it does about the epithelial cells. In this inactive state the mesenchyme and connective tissue cells may remain apparently indefinitely unless new medium is added to the culture.[2]

In the earlier studies of the tissue culture it has also been noted that the rate of activity of any given tissue is inversely proportional to its age. About equal size fragments of the heart muscle of chick-embryos the rate and extent of migration becomes less and less as these fragments are taken from older and older embryos.

Directly associated with this decrease in the rate and extent of migration the degeneration becomes less and less extensive and growth finally ceases. Together with these changes the latent period before any activity is noted in the cultures, increases. About fragments of a 5 day old chick-embryo heart, migration may begin within 1 hour of the time the cultures are prepared. About a simple fragment of a 10 day old chick-embryo heart, no activity is seen before 6 to 12 hours and about a 15 day old chick-embryo heart, before 12 to 18 hours.

At first I thought that these changes were to be related directly to an aging of the cells. It soon became evident, however, that this was not true. It was to be related rather to a gradual development of intercellular substances and blood vessels and a corresponding separation of the cell. The rate and extent of migration, the growth or degeneration of any tissue is proportional to the original cell-density of the fragment whether food and oxygen are maintained or not. The cancer cells of the cultures are not different from the normal cell. The cells from fragment of cellular granulation tissue react like those from equally cellular fragment of the embryo. The cells from cellular fragment of a malignant round cell-sarcoma react like the mesenchyme cells from the young embryonic tissue while those from a fibro-sarcoma react like the cells from older embryos in which the intercellular substance and blood vessels are the same as that of the fibro-sarcoma.

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[2] Burrows, M. T., *XVII Int. Cong. Med., Gen. Path. and Path. Anat.*, London, 1913, 217

The only difference that I have been able to find between the cancerous tissue and tissue of young embryos is a richer supply of reserve nutrient substances in the embryonic tissues. The cancerous tissues have little of this reserve. Extracts of cancerous tissues stimulate growth in other cells like extracts of embryonic tissue but they do not prolong it.

While it is true that many authors have assumed that the disappearance of independent growth in embryonic life is the result of an aging of the cell, the best work of this and the last century has shown that this is not true. As Wilson[3] clearly states, growth, differentiation and function are changes not determined by the cell but by more general formative forces or stimuli. Ribbert had fully appreciated this fact in relation to cancer. As he states the remarkable power of tissues to regenerate shows clearly that the cells of the fully developed organism have not lost their property of growth. The growth in cancer Ribbert did not find unique. He thought it could be nothing more than the response that any cell may undergo under the influence of a proper change in the environment.

The difficulty which confronted Ribbert and his school has been the finding of the nature of the environment suitable for this change. The tissue culture as I have already pointed out has aided much in this search. In previous studies[4] I had noted that embryonic heart muscle cells may respond like simple migrating mesenchyme cells, actively growing cells or normally rhythmically contracting cells in the culture. Each of these responses is to be related in each case to a specific environment. The rhythmically contracting cells are stretched through a serum cavity between a surface of the medium in the tissue fragment and the end of a band of fibrin. This same cell removed from these contacts and laid against a fibrin-fibril stretches out to become a simple spindle shaped connective tissue-like cell. When placed at the surface of the medium near a cellular fragment of tissue it flattens to an irregular polygonal or spindle shape, grows actively and divides by mitoses.

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[3] Wilson, E. B., *The Cell in Inheritance and Development*, 1906.

[4] Burrows, M. T., 2nd Pan Am. Sci. Cong., Washington, D. C., Sect. VIII, Pt. 2, 494

In a further analysis of muscular contraction I showed that this whole phenomenon can be explained as a differential surface tension phenomenon established by specific contacts of the cell with a heterogeneous environment.[5] In the same manner it can be shown that all functional activity in the organism, like the shape and arrangement of the cell, can be directly related to the immediate environment surrounding the cells.[2]

From these later observations it became evident that growth is not something peculiar to the younger cells. It is a reaction which may be the property of any fixed tissue cell when placed in the proper environment. The environment suitable for such growth is one where the cells are crowded together and the circulation is reduced.

In this regard, as it is evident, body cells are not different from many unicellular organisms. Wildiers,[6] 1902, had shown that single yeast cells will not grow in a large bulk of medium. For growth to take place a certain bulk relation must exist between the medium and the yeast. Webb, Williams, and Barber[7] found that a single tubercle bacillus or a single spore of anthrax will not infect an animal. Several must be inoculated. So in the same manner Robertson[8] has pointed out that the growth rate of paramecium increases with the number of organisms present in the medium.

Wildiers had also noted that this inability for the single yeast cell to grow is due to the lack of some product liberated by these cells. Extracts of yeast added to the medium allow the active growth of a single cell to proceed.

In the early history of a tissue culture Carrel and I[9] had already noted that an active growth stimulant may be readily extracted from fresh embryonic and cancer-tissue. This stimulant is not present in most adult tissues but as the above experiments show this stimulant must develop in these tissues when they are cut away from the body and placed in the culture. Again these experiments indicate that the amount of this stimulant generated is directly proportional to the original cell-content of the fragment. In proof for this I have extracted many of these fragments with isotonic sodium chloride solution after they had remained for a time in the culture and recovered the stimulant as indicated by the action of these extracts on other cells.

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[5] Burrows, M. T., *Am. J. Physiol.*, 1917-18, xlv, 556.

[6] Wildiers, *E. La Cellule*, 1902, xviii, 313.

[7] Webb, G. B., Williams, W. W., and Barber, M. A., *J. Med. Res.*, 1909, xx, 1.

[8] Robertson, T. B., *Biochem. J.*, 1921, xv, 596.

[9] Carrel, A., and Burrows, M. T., *J. Am. Med. Assn.*, 1910, lvi, 32.

While these experiments of Carrel's and mine were interesting in showing that the active independent growth of cells in early life and in cancer may be the direct result of the presence of a growth stimulating substance in these tissues, they became of little importance in that they failed to show the source of this substance.

In my earlier experiments with the tissue culture I had already shown that autolysis as it occurs from the absence of oxygen does not liberate a growth stimulating substance. Extracts of such tissues retard or completely inhibit all cell activity. The growth of nerve fibers is strikingly inhibited about the larger fragments of the neural tube while it is most active about smaller ones.[10] In a recent paper before this Society I have again shown that the development of the stimulus for growth is dependent on the presence of oxygen, while the growth reaction itself like muscular contraction (Fletcher) is not dependent on this gas. The cells from fragments of the heart muscle of 5 day old chick-embryos will show a limited growth in the absence of oxygen. The cells in older fragments demand oxygen for the same limited growth. The amount demanded by these older tissues is directly proportional to their age and the latent period for their growth in an ample oxygen supply.[11]

Drew[12] has recently stated that autolyzing tissue liberates a growth stimulating substance. This idea as it is evident from the above observation is wholly unfounded. His own experiments also disprove his contentions. He extracted the stimulus from a kidney incubated for one hour. This is not long enough for autolysis to set in.

From the work cited above on the tissue culture it becomes evident that growth in body cells is directly related to a crowding of the cells or to the establishment of conditions suitable for the accumulation of certain products of their normal metabolism about them. The earlier experiments of Carrel and mine had already shown that the stimulating substance for the growth of cells is soluble in salt solution. The studies reported in paper No. 1 of this series, indicate clearly that it may be washed away from the tissue with serum. These observations show that this stimulating substance is a product of the normal metabolism of the cell. These cells can grow only when they are crowded together in an environment where this product of their metabolism is not rapidly removed from them.

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[10] Burrows, M. T., *J. Exp. Zool.*, 1911, x, 63.

[11] Burrows, M. T., PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 133.

[12] Drew, A. H., *Brit. J. Exp. Path.*, 1923, iv, 46.

49 (2281)

Studies on cancer.

III. Cellular growth and degeneration in the organism.

By MONTROSE T. BURROWS.

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As it is well known an active independent growth of cells in the body is peculiar alone under normal conditions to the earlier periods of development. At first this growth is quite generalized. Later it becomes localized, first in one part and then another. In man it ceases entirely with the laying down of the last kidney tubule and glomerulus at about 10 days after birth. Subsequent to this time all growth is merely the enlargement of pre-formed organs and tissues. It is like the hypertrophies and hyperplasias of later life.

In later life it is again well known that hypertrophy and hyperplasias are related directly to the functional activity of the part. Increase the work of the heart, it grows. Decrease its activity, it atrophies. So, in the same manner, the removal of a part of any organ or another organ of the same kind leads to an active enlargement of the remaining parts. This enlargement continues to the re-establishment of a certain size which corresponds to the functional demand made upon the part.

As a moderate increase in the functional demand made upon an organ or a part is associated with the growth of that organ or part so further or excessive work is associated with a degeneration of these same cells. Such a degeneration is also seen in certain structures during development. The pronephros and the metanephros of higher animals suffer such changes as they give way for the development of the kidney.

Cancer represents a return of the property of independent active growth to certain cells or rather to groups of certain cells in the organism. This independently growing tissue also often suffers extensive degenerative changes in part. While these degenerations resemble ordinary autolytic changes they differ from those seen in the infarct, for instance, in that they are not harmful for the organism. The disappearance of the pronephros or the metanephros does not disturb the nutrition of the whole; so it has been shown that large tumors may disappear in a few days without evident toxic symptoms to the animal.[1]

Closely associated with this loss of independent active growth in the developing organism marked morphological and chemical changes also take place in the tissue. The various cells peculiar to the different organs and tissue are differentiated. The blood vascular system develops. The cells become separated by a rich vascular network and by a deposit of extracellular reticular fibres, the connective tissue fibrils. In early embryonic life, the cells are closely packed together. The number of capillaries per unit cell-area is small. In later life one sees great variation in the number of cells per unit area in any tissue. In the most cellular tissue of this period all cells are closely beset with capillaries. The cells of the liver for instance are separated into columns no more than 2 cells thick. The circulation is active in these capillaries. These conditions are in contrast to the slow sinisoidal circulation of the embryonic liver.

Such an arrangement of cells peculiar to the adult is strikingly different, therefore, from the dense cells-masses of cancer and early life. Cancerous tissue is peculiar not only in that it grows actively and independently of the whole or of any functional demand made upon its cells but it again, like the tissues of early embryonic life, is a densely cellular tissue. It is peculiar not that its cells are like the embryonal cells—they are not—but that they are arranged in dense masses. They have lost in many instances the shape peculiar to them in the normal tissues. Otherwise they are not embryonal. The cells of the cancer of the skin are skin epithelial cells. So the cells of the sarcoma are connective tissue cells. The only demonstrable change they have suffered is in their arrangement, their ability to grow and their shape.

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[1] Ewing, J., *Neoplastic Diseases*, 2nd ed., 1922, 141.

For many years it has been known that increased functional activity in the organism is associated with an increased circulation to the part. Growth quite the reverse may follow a decrease in the circulation. The clubbing of the fingers with disturbance in the general circulation illustrates clearly this fact. In an unpublished paper on the circulation of the nail I have found that the circulation of the growing bed is very much less than along the whole of the outer parts of this structure.[2] The entire growth of the developing nervous system takes place along the central canal. The blood vessels invade the outer peripheral portions. In the light of these facts it may not be surprising, therefore, that the heart hypertrophies when it is overloaded, its cavities dilated and its walls compressed. Such compression of the wall must impede the circulation about the muscle fibres.

Correlating the above observations of the cultures, cited in the two preceding papers, with conditions as they exist in the body it became evident, therefore, that the disappearance of an active independent growth of cell in the developing organism may be none other than the direct result and orderly laying down of intercellular materials and the development of a rich vascular supply. Cancer looked at in this sense may be the direct result of any change in the organism which leads directly to the accumulation of dense masses of cells free from intercellular substances and having a reduced blood supply or to an orderly disappearance of the normal vascular supply.

To give further proof for this conception it became of interest to study more carefully and quantitatively the action of this growth stimulus on the cells. Devloo[3] in analyzing the active growth stimulating substance noted by Wildiers in cultures of yeast came to the conclusion that it is either a phospholipin or is associated with or dissolved in lecithin. In my studies of the cells of higher animals I have identified a substance liberated by these cells which is an active blood coagulant and otherwise has many of the physical properties of a phospholipin. This substance I have been able to quantitatively show produces the energy for the migration of these cells, for the development of tension or the energy for muscular contraction and combines to form the extracellular fibrils of connective tissue. In growing tissues it is formed in excess and disappears in the presence of food so it may be retained in the reaction forming protoplasm of certain parts of the cell. This is not the stimulating substance. The stimulating substance is soluble in normal saline solutions and in body fluids. It acts in lower concentrations to the excessive formation of the energy producing substance. In higher concentration it acts to break down the proteins of the cells. The degeneration produced by this substance is histologically like autolysis. Physiologically these degenerating cells act quite differently. They are utilized as food by the other cells while autolyzing tissue inhibits activity in these cells. The degeneration seen in the centers of the small fragments in the culture and later in other cells of the cultures well supplied with oxygen and in the crowded cell area of cancer is of this type. It is not a primary autolysis. It is the result of a greater accumulation of that same substance which in lesser concentration leads to an active growth of these cells.[4]

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[2] Proirer and Charpy also note this fact (*Traité de Anat. hum.*, v, 184).

[3] Devloo, R., *La Cellule*, 1907, xxiii, 359.

Again I have not found any evidence to show that this stimulating substance combines in any of the reactions of the cell. It acts as a catalyzer of other reactions of the cells. Whether it is a product of the same reactions which it accelerates I have not fully determined.

Its activity at all times is wholly dependent on its concentration. Its concentration at all times is directly proportional to the rate of its production and indirectly proportional to the avenues for its escape by way of the blood stream.

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[4] Burrows, M. T., *Mo. State Med. J.*, 1923, xx, 145.

50 (2282)

Studies on cancer.
IV. Factors regulating the production of cancer in the organism.

By MONTROSE T. BURROWS.

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Recent and older careful studies of cancer have shown that this disease may be induced by a variety of substances and conditions; such as coal tar, various lipoid solvents, arsenic, various animal parasites, X-ray, radium, etc. In mice it occurs most frequently in certain families (Maude Slye). In man it may arise in certain congenital defects and tumors and in old inflammatory processes.[1]

These same studies have further indicated that cancer unlike the inflammatory processes when once induced is not dependent any longer upon these original exciting agents. Cancers once induced by coal tar may be transplanted through many generations of animals.[2]

From observations cited in the three preceding papers of this series it is evident that an independent growth of body cells is dependent on a crowding of these cells together and a relative decrease in their blood supply. It became thus of interest to see what might be the nature of the changes induced by these various excitants of cancer. This work was just begun three years ago. It has been described in part in other papers.

Ross[3] in discussing the action of these various substances which lead to cancer thought them direct excitants of cell division. It seemed evident to me from my studies of the factors regulating cellular growth in the organism that their action might be otherwise. Fischer[1] many years ago had already shown that when drops of olive oil containing Sharlach R are introduced just beneath the epidermis the epithelial cells are attracted to these drops. These cells surround the drops of oil to form dense cell masses not unlike those of cancer. Jorstad[4] working in my laboratory has found that coal tar acts in the same manner. Drops of this substance attract the cells to them and away from their blood vessels and thus they build a dense mass of cells peculiar to the normal organism and with a relatively poor blood supply. These masses as they grow larger become autonomously growing systems.

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[1] Ewing, J., *Neoplastic Diseases,* 2nd ed., 141.

[2] Woglom, W. M., and Murray, J. A., 7th *Sci. Rep. Imp. Can. Res. Fund,* 1921, 45.

[3] Ross, H. C., *J. Can. Res.,* 1918, 111, 321.

Whether the coal tar in itself may induce division in these cells has not been fully determined. In Jorstad's preparations no division-figures were seen until the masses themselves were of a size consistent with such changes as deduced from their occurrence in the cultures.

In the same manner I have found that the tape-worm described by Bullock and Curtis[5] attracts connective tissue cells to it. It does not induce the densely fibrous capsule peculiar to the ecchinococcus for instance but a very cellular one. The spirotera of Febiger[6] shows a similar attraction for the epithelial cells of the stomach.

These substances act, therefore, quite differently from those which produce inflammation. These latter substances induce primarily an exudate from the blood vessels. The cells react secondarily to organize and become scattered in the exudate. The cancer producing substances act on the cells directly to attract them away from their intercellular substances and blood vessels. The tissue built in the former case is a richly vascular tissue with a large bulk of intercellular substance. In the latter instance it is a densely cellular tissue poor in intercellular substances and in blood vessels.

As Ross has clearly pointed out it is not injury that produces cancer, it is the action of specific substances. Our studies have indicated that these substances attract cells to them, away from their intercellular material and blood vessels and form a dense local mass free from ready avenues of escape for the soluble products of their normal metabolism.

A soluble stimulus placed in the tissue can have no such effect. It must escape by way of the blood stream. It is a viscid mass which remains fixed and attracts cells to it that act in this regard, like certain animal parasites, thick drops of coal tar, drops of olive oil or better, olive oil containing substances like Sharlach R. One of the Swedish observers has noted that only drops of coal tar having a high boiling point induce cancer.

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[4] Jorstad, L. H., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, ?

[5] Bullock, F. D., and Curtis, M. R., *Trans. N. Y. Path. Soc.*, 1920, xx, 149.

[6] Febiger, J., *J. Can. Res.*, 1919, iv, 367.

The exudates of chronic inflammatory processes attract cells to them as Hertzler's[7] studies have indicated and the work of the cultures have proven. In the body these same conditions also are associated with the development of blood vessels and exudates become coagulated and deposited as extracellular fibrils between the cells. Primarily these processes lead away rather than towards any cancerous process. The epithelial cells of the glands and other parts do not, however, react readily to these exudates. They become imbedded in this mass of newly formed connective tissue. Later this mass of connective tissue changes. The blood vessels become obliterated. The connective tissue becomes hyaline. The cells degenerate. With this change in the blood supply the separated epithelial lined ducts suffer atrophy to a greater or less extent. At the same time their environment becomes more suitable for an active proliferation. Carcinoma is a disease of old age. It develops frequently in the epithelial cells of the skin which are undergoing atrophy. As it is well known the breasts of women and probably other tissues suffer periodic stimulation from the sex glands and other internal secreting glands. As it is evident from the above laws of growth laid down, such atrophying epithelium must respond quickly to any stimulant for cellular growth or as the culture-experiments indicate such a tissue may begin to grow independent of such a stimulation. To prove this it becomes of interest to study the effect of a soluble stimulant upon tissues of adult animals. The stimulant used was the one produced by the tissue as noted above. It was extracted from the Jensen sarcoma with normal saline solution. The solutions of this stimulant were made sterile of sarcoma cells by passing them through a Berkfeldt filter. When introduced into the normal skin of an adult rat this solution had no noticeable effect. It was evidently removed by the blood stream. When injected into areas of skin suffering pressure atrophy this solution produced carcinomatous proliferation[8]

We have not investigated as yet the action of X-ray and radium. Members of families of mice which frequently develop cancer have also not been investigated. From the above observations it seems evident, however, that cancer may result from any condition or substance which can build a dense mass of cells in the organism; to a proper reduction in the blood supply of any cellular tissue so that the growth stimulus may accumulate and the nutrition remain unchanged. The simple pigmented mole is such a mass of cells. These moles as it is well known may become malignant after injury or disturbances in their blood supply or in the course of their normal development in the aging organism. Such age-changes are associated with a decreased vascularity and atrophy of the superficial tissue. The relation between senile keratoses and cancer has long been recognized by dermatologists.

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[7] Hertzler, A. E., *The Peritoneum*, 1919, 1, Chap. V.

[8] Burrows, M. T., *Mo. State Med. J.*, 1923, xx, 145.

Further studies of the mechanism of cell-division which have just been completed in the laboratory have shown that an orderly division of cells to form columns and to line the ducts of glands is directly related to the circulation and position of the blood vessels. Decrease this circulation and stimulate the cells, lateral division and dense masses may develop.

While it is true that many authors, such as Child,[9] have assumed that differentiation is the normal outcome of all cellular growth it is possible to show quite definitely as I have indicated in an accompanying article[10] that is not true. Such a cellular system as cancer once developed in the animal organism reproduces itself.

The striking peculiarity of body cells which distinguishes them from unicellular organisms is not in that part of their general organization which has to do with their growth and function but it is their inability to utilize the crude materials from nature as food. They again have no protection from external influences in the form of membranes. Much more crowding is necessary to obtain growth in body cells than in the unicellular organism.

These body cells cannot even burn sugar without the intervention of the pancreas so the whole developing animal depends absolutely for its nutrition on the yolk and the mother. True yolk is formed by the parent body. In man as in animals all the essential organs are formed before it is possible to remove the animal from its yolk or its mother. The cells are nourished always by foods which have first passed through a fully formed organism.

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[9] Child, C. M., *Senescence and Rejuvenescence*, 1915.

[10] Burrows, M. T., read before the Southern Med. Soc., Washington, Nov. 15, 1923.

Differentiation like growth is not a primary phenomenon in all periods of life but in the early stages. It is hastened as many previous experiments have shown by removing parts of the yolk or by accelerating the metabolism of the developing organism through the feeding of thyroid.

Cancer as we have seen it, therefore, may be the normal outcome of any substance or condition capable of building a densely cellular tissue in the organism associated with an orderly decrease in the blood supply. It is not the result of any primary changes in the cell but to a specific rearrangement of cells, intercellular substances and blood vessels in the organism.

ABSTRACT OF COMMUNICATIONS.

Peking Branch.

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51 (2283)

Experimental infection of culture rats and mice with the common intestinal amebae of man.

By JOHN F. KESSEL (by invitation).

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The present investigation was undertaken in order to check on the previous reports in which attempts were made to infect rats and mice with *Endameba dysenteriae* and also to infect these rodents with the other common intestinal amebae of man.

Infections of five species of human intestinal amebae, *Endameba dysenteriae, Endameba coli, Councilmania lafleuri, Endolimax nana*, and *Iodameba büttschli* have been experimentally transferred to rats by feeding them human feces containing cysts of these amebae. Mice have been experimentally infected with *Councilmania lafleuri* and *Endameba dysenteriae.*

A greater percentage of young amebae-free animals than of old amebae-free animals or of young animals known to possess an infection of amebae common to the rat and mouse have become infected with these five amebae common to man.

Infections of *E. dystenteriae, E. coli,* and *C. lafleuri* have been experimentally transferred from rats harboring experimental infections of these amebae to other young amebae-free rats by feeding them the feces of the infected rats.

The amebae transferred from man to the rodent host have, in every case presented no apparent racial or morphological change during the period of the experiment. The data acquired in this investigation give interesting information on the questions of (1) morphological constancy of parasitic Protozoa, (2) host specificity of parasite, (3) the possibility of rodents acting as carriers of amebiasis.

Infections of *E. dysenteriae* in rats and mice apparently assumes a chronic form rather than an acute form as is reported in kittens.

52 (2284)

A modification of Duboscq-Pellin colorimeter for bicolorimetric work as in colorimetric pH determination without buffer mixtures.

By HSIEN WU.

[*From the Laboratory of Physiological Chemistry, Peking Union Medical College, Peking, China.*]

A colorimeter in which the combined color of two solutions is used as the standard is required for some purposes. The best illustration is the colorimetric determination of pH without standard buffer mixtures, where the color of the indicator in the unknown solution is matched with the combined color of an acid solution and an alkaline solution of the same indicator. Knowing the ratio of the depths of the two color solutions of the same concentration or the ratio of the concentrations of the solutions of the same depth, the pH of the unknown solution which matches with that of the combination can be calculated by means of an equation derivable from the mass action law and the theory of indicators or by referring to a curve determined experimentally.

Various devices have been used with more or less success for superimposing the colors by Bjerrum[1], Barnett and Chapman,[2] Barnett and Barnett,[3] Gillespie,[4, 5] and Myers.[6] But since the Duboscq-Pellin colorimeter is more widely used than any other type of colorimeter, on account of the excellent optical system it possesses, it seems desirable to modify this instrument so that it can be used for bicolorimetric as well as for monocolorimetric work. Such a modification would also avoid the unnecessary duplication of colorimeters.

The modification here described consists essentially in placing on one side of the colorimeter above the original stage a new cup, which is carried on a new stage fitted with an independent rack, pinion and vernier. Thus while one side of the instrument remains unchanged the other is fitted with two cups, each with its own adjustment and vernier, the upper of which is an entirely new addition and requires a new slit in the brass back of the colorimeter. This slit is 5 mm. above the original slit and is about 60 mm. long. The rack and pinion construction is identical with that of the original instrument.

The upper cup has the same capacity as an ordinary 50 mm. Duboscq cup, and the original cylinder is used. The metal casing extends almost to the top of the cylinder and is made from a brass tubing by cutting a window on each side, leaving two cylinders joined together by a pair of bands about 10 mm. wide. The lower cylinder is 15 mm. and the upper cylinder 8 mm. long. The windows facilitate the operation of cementing the glass parts and permit observation of the plunger when the cup is in position. The lower end of the casing is beveled to fit a similarly beveled glass disc, which is 5 mm. thick and projects about 1 mm. beyond the metal casing.

This casing is suspended from a stage rigidly attached to the rack and pinion. The upper part of the casing is provided with two pairs of keys, one pair above each metal band, by means of which it is held rigidly by the stage. The stage is provided with slots through which the keys can pass and by turning the casing through 90 degrees it can be instantly detached or placed in position.

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[1] Bjerrum, N., *Die Theorie der alkalmetrischen und azidimetrischen Titrierung.* Stuttgart, 1914.

[2] Barnett, G. D., and Chapman, H. S., *J. Am. Med. Assn.*, 1918, lxx, 1062.

[3] Barnett, G. D., and Barnett, C. W., PROC. SOC. EXP. BIOL. AND MED., 1920-21, xviii, 127.

[4] Gillespie, L. J., *J. Am. Chem. Soc.*, 1920, xlii, 742.

[5] Gillespie, L. J., *J. Bact.*, 1921, vi, 399.

[6] Myers, V. C., *J. Biol. Chem.*, 1922, liv, 675.

The lower cup is similar to the ordinary Duboscq cup, but it is a little shorter and larger in diameter. The bottom of the metal casing is provided with a ring which fits accurately into the groove of the original stage. The casing is 52 mm. in outside diameter, 49 mm. in inside diameter, and 20 mm. high. The glass disc for the bottom is 2 1/2 mm. thick. The cylinder is made from pyrex tubing 48 mm. in diameter and 2 mm. thick.

The cup for the other side of the colorimeter is an ordinary Duboscq cup. Only the original disc is replaced by two discs, one on top of the other. These discs are of same thickness and made of the same glass as the discs for the upper and lower cups of the other side.

The vernier for the upper cup is so placed that it reads zero on the original scale when the plunger touches the bottom of the cup. The zero point of the lower cup is determined when the plunger and the bottoms of the two cups are in contact. The zero point of the cup on the other side is determined in the usual manner.

A preliminary testing of the instrument with phenol red has been made. For producing the acid and alkaline colors a 0.7 per cent KH_2PO_4 solution and 0.1 per cent Na_2CO_3 solution respectively were used according to Hatfield.[7] Clark and Lub phosphate mixtures giving pH values from 7.0 to 8.0 were used as unknowns. The color solutions were prepared by adding 1 cc. of 0.008 per cent solution of phenol red to each 10 cc. of the buffer. The acid solution was placed in the inner cup and the alkaline solution in the outer cup. The unknown solution was placed in the cup on the other side. The cup for the unknown and the lower cup were set at 20 + Z. mm. (Z = Zero error). Perfect matches were obtained by moving the upper cup.

The result of the testing with phenol red is shown in the accompanying table. If the readings are plotted against pH values a smooth curve is obtained. The value of pK for phenol red calculated by means of the equation

$$\mathrm{pH} = \mathrm{pK} + \log \frac{\text{Depth alkaline color}}{\text{Depth acid color}}$$

is 7.76 at 25-28° C. This agrees well with the result of Barnett and Barnett, who obtained the value 7.77.

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[7] Hatfield, W. D., *J. Am. Chem. Soc.*, 1923, xlv, 940.

pH of buffer	Reading of acid color	Reading of alkaline color (by difference)	$\log \frac{\text{Depth alk. color}}{\text{Depth acid color}}$	pK
	mm.	mm.		
7.0	17.1	2.9	1.23	7.77
7.2	15.6	4.4	1.45	7.75
7.4	13.9	6.1	1.64	7.76
7.6	11.8	9.2	1.89	7.71
7.8	9.7	10.3	0.03	7.77
8.0	7.6	12.4	0.21	7.79
			Average	7.76

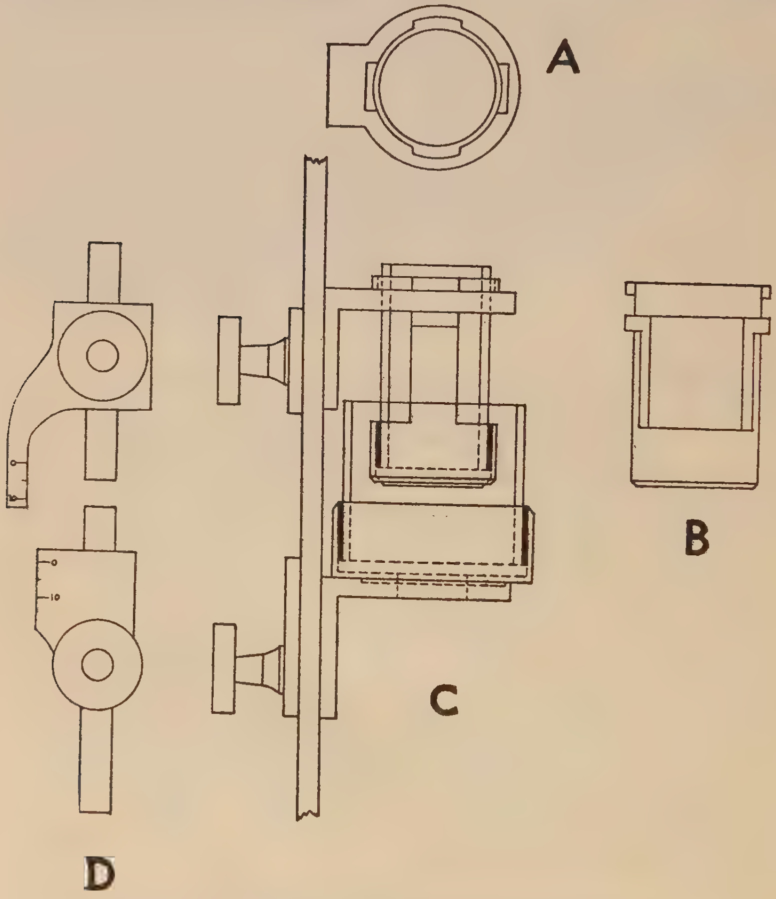

Diagram of modified Duboscq-Pellin colorimeter for bicolorimetric work.
A. Top view of upper cup casing and stage, showing slot and key arrangement.
B. Front view of upper cup casing.
C. Side view of both cups in position.
D. Back view of the colorimeter showing the relation of the slits and verniers.

53 (2285)

Variations in alkali reserve and acid-base balance in tetany.

By E. W. H. CRUICKSHANK.

[*From the Department of Physiology of the Peking Union Medical College, Peking, China.*]

The idea that tetany was a condition associated with alkalosis originated from the high figures for the CO_2 combining power of the blood obtained in tetany occasioned by gastric lavage and was strengthened by the statement that tetany may be accompanied by a pH as high as 7.8. As a result of work carried out here two years ago, the conclusion was reached that the variation in the pH to the alkaline side of normal need not necessarily be great, and that such a variation was undoubtedly followed by a fall to the acid side of the normal for the animal under observation.

To investigate changes in the acid-base balance by determining values for the pH, $[CO_2]$, $[H_2CO_3]$ and $[BHCO_3]$ of the blood, CO_2 dissociation curves were drawn for the blood of normal and thyroparathyroidectomized dogs, the determinations on the latter being made on the three consecutive days following the operation. The pH was calculated from the equilibration data by use of the Hasselbach formula and thus a curve approximately linear in character was obtained in which $[CO_2]$ was related to pH. The pH for the blood under consideration was then found by interpolation upon this curve of the total $[CO_2]$ value of the blood as drawn. To determine changes in the alkali reserve the value of the $[CO_2]$ for the blood as drawn was extrapolated to the value which would obtain at the CO_2 tension necessary to maintain the pH at the normal figure for the animal. From the value thus found the $[BHCO_3]$ was calculated,

$$[BHCO_3] = \frac{[CO_2] \times R}{(R + 1)}$$

R being equal to the ratio $[BHCO_3] : [H_2CO_3]$; the change in the base content of the blood then indicated the excess of base over acids other than H_2CO_3.

The results so far have been remarkably uniform and indicate that the form which the associated blood condition may assume depends largely upon the degree of the tetany produced. Within the first twenty-four hours after the operation the condition is one of alkalosis; the pH rises on an average of 0.154, this being due to an uncompensated CO_2 deficit. This stage is marked by an absence of any of the signs of acute tetany, it may be regarded as a quiescent or primary phase of the disease, while the subsequent stage which is one of acidosis, marked as it is by definite tremor, violent spasmodic twitching of the muscles of the head and lower jaw, spasticity of leg muscles and rapid respiration, may be regarded as the acute phase, or the tetany proper.

The loss of base begins in the first stage and continues progressively throughout the second, while the loss of volatile acid, which is marked in the first stage far exceeding the loss of base, is partially recovered from and may either remain stationary or fall again with the development of acute tetany. The alkali reserve which may be increased in the first stage begins to fall with the onset of the acute condiiton and continues to fall rapidly until the death of the animal. The point at which the pH again reaches and passes the normal is indicated very approximately, in a graphic representation of these results, by the point at which the acid : base ratio again becomes balanced although on a lower level.

As an example of the results obtained, in which the changes in acid, base, and alkali reserve are presented as a percentage of the normal, the following table may be given:

Normal		Tetany 24 hrs.	Tetany 48 hrs.	Tetany 72 hrs.
pH	= 7.440	7.603	7.430	7.360
$BHCO_3$	= 16.830	26.15 per cent —	21.03 per cent —	36.20 per cent —
H_2CO_3	= 0.840	45.11 per cent —	9.54 per cent —	15.48 per cent —
Alk. res.	= N.	0.24 per cent +	24.90 per cent —	45.35 per cent —

54 (2286)

Variations in the distribution of carbon dioxide and chlorides in the cells and plasma of blood in tetany following thyroparathyroidectomy in dogs.

By R. G. BIRRELL and E. W. H. CRUICKSHANK.

[*From the Department of Physiology of the Peking Union Medical College, Peking, China.*]

MacCallum and co-workers have drawn attention to the fact that there is a decided loss of chlorides from the blood in gastric tetany, while Tisdall has demonstrated a similar loss in parathyroid tetany. It is known that the chloride content of the blood, small though it is, does not readily suffer change and it seemed to us that while there is no marked loss of chloride from the whole blood in parathyroid tetany there may be, in the acute condition which has been so marked a feature of our thyroparathyroidectomized animals, some definite alteration in the distribution of the chlorides in the plasma and cells of the blood. To determine this, and if possible relate it to changes in a similar carbon dioxide distribution, we have undertaken the present investigation.

It should be noted that by mild tetany we mean that condition of the animal immediately following operation, in which there may be no manifest signs of tetany or at most fine muscular tremor, felt most easily over the shoulder girdle of the animal. By acute tetany on the other hand is meant the severe and at times distressful condition which usually commences the second day following operation and is marked by severe spasmodic twitching of the supra-orbital and masseter muscles, spasticity of the legs, rapid respiration and profuse salivation, as described in a previous paper by one of us (E. W. H. C.).

Estimations of carbon dioxide were made by the Van Slyke method, of chlorides by the Van Slyke-Donleavy method. The results upon four dogs are reported; the normal results being compared with those obtained upon the four dogs 24 hours after operation, at which time all were in a stage of mild tetany; upon three of the dogs 48 hours after operation, and upon one dog 72 hours after operation; these latter were all cases of acute tetany.

Results: It will be seen from the table here given that the CO_2 content of the plasma and cells forms 74.2 and 25.6 per cent of the total CO_2 content of the whole blood. In mild tetany, while there is a slight increase in the plasma CO_2, there is a

	Whole Blood			Plasma			Cells		
	Volume per cent	Per cent of whole blood	Percentage change in whole blood	Volume per cent	Per cent of whole blood	Percentage change in plasma	Volume per cent	Per cent of whole blood	Percentage change in cells
Normal	43.47	100	——	32.28	74.2	——	11.13	25.6	——
Mild tetany	43.61	100.3	+ 0.3	34.86	80.2	+ 8.0	8.75	20.1	—21.4
Acute tetany	35.11	80.8	—19.2	28.65	65.9	—11.2	6.41	14.7	—42.4

BLOOD CHLORIDES

	Whole Blood			Plasma			Cells		
	Gm. per 100 cc. of blood	Per cent of whole blood	Percentage change in whole blood	Gm. per 100 cc. of blood	Per cent of whole blood	Percentage change in plasma	Gm. per 100 cc. of blood	Per cent of whole blood	Percentage change in cells
Normal	0.599	100	——	0.422	73.9	——	0.156	26.1	——
Mild tetany	0.580	96.8	— 3.2	0.453	75.7	+ 2.6	0.126	21.1	—19.9
Acute tetany	0.584	97.5	— 2.5	0.469	78.2	+ 6.1	0.114	19.1	—26.9

definite loss of the cell CO_2, there being no change in the total amount. In acute tetany, however, there is a definite loss of CO_2 from the blood amounting to a loss of some 20 per cent. The point of note here is that the cell loses a much greater percentage of its normal content than does the plasma.

With regard to chlorides it is evident that they undergo little change in the whole blood or plasma while from the cells there is a definite loss of chlorides even in mild tetany. That some toxin is at work destroying cellular function is indicated. As to the nature of the mechanism involved we can at present offer no explanation.

55 (2287)

A method for maintaining stability in reaction of solutions during sterilization.

By O. H. ROBERTSON, SHU-TAI T. WOO, and RICHARD H. P. SIA.

[*From the Department of Medicine of the Peking Union Medical College, Peking, China.*]

Of the various means employed to prevent the marked changes in H-ion concentration which solutions undergo during sterilization, the use of relatively large quantities of Na or K phosphate has proved the most satisfactory. While this method may be suitable for certain types of solutions, the presence of large quantities of phosphate in solutions used for intravenous injection, and serological procedures, is undesirable. A very much smaller quantity of phosphate added to an adjusted solution after sterilization will maintain a stable reaction satisfactorily, but the disadvantage of adjustment after sterilization is obvious.

An attempt to adjust and buffer certain solutions before sterilization with an amount of phosphate as low as that found in the circulatory blood plasma, led to recognition of the fact that the chief variable in the solutions tested was their CO_2 content. The degree of CO_2 exchange between fluid and surrounding air during heating determined the reaction of the solution afterwards. Elimination of this variable by the use of CO_2 free water and CO_2 free reagents made it possible to autoclave 0.9 per cent NaCl solution, dilute gelatin solutions and water without changing, to more than a slight degree, their initial reaction. The method employed was as follows:

The water employed was twice distilled and rendered CO_2 free by boiling just before use or preferably by bubbling CO_2 free air through it for 24 hours. After the addition of the solute 1 per cent M/7.5 H_3PO_4 (previously boiled and kept in a bottle stoppered with a soda-lime trap) was added. Next, M/7.5 NaOH, prepared in the same way as the H_3PO_4, was added in sufficient quantity to bring the solution to the desired reaction. The solution was autoclaved at 14-15 lbs. steam pressure, care being taken not to let the steam escape during sterilization. The autoclave was not opened until the following morning.

Solutions prepared in this way showed a slight but constant change toward acidity as a result of sterilization. The degree of change seemed to depend on the initial reaction of the solution before sterilization. Solutions adjusted to pH 7.0-7.5 were found after autoclaving to be 0.1 to 0.2 of a pH unit lower than the initial reaction, while with reaction of pH 7.6-8.0 before autoclaving the change was found to be slightly greater, 0.2 to 0.4 of a pH unit. The change in pH during sterilization was found to be greater in the more alkaline solutions. The reaction then remained practically constant over a period of two to three weeks. Since the phosphate content of the above solution is about the same as the concentration of the inorganic phosphoric acid compounds found in the normal human blood plasma, physiological salt solution prepared in this way should prove suitable for intravenous injection.

ABSTRACTS OF COMMUNICATIONS.

Western New York Branch.

Ninth Meeting.

Buffalo, New York, November 10, 1923.

56 (2288)

Comparative study of the blood sugar concentration in the liver vein, the femoral artery and the femoral vein during insulin action.

By CARL F. CORI, GERTY T. CORI and HILDA GOLTZ (by invitation).

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

A new method has been developed for the collection of blood from the liver vein, whereby admixture of blood from the *vena cava* is avoided. The puncture of the liver vein requires no narcosis of the animal and can be repeated several times.

The normal sugar values for different blood vessels obtained on rabbits which were previously starved for 24 hours were as follows:

1. For the difference in blood sugar concentration of the liver vein and the neck vein: 28 mg. (average of 9 experiments).
2. For the difference in the blood sugar concentration of the femoral artery and femoral vein: 8 mg. (average of 20 experiments).
3. For the difference in blood sugar concentration of the liver vein and the femoral artery: 23 mg. (average of 9 experiments).

The following two questions were studied:

1. Does the liver show a diminished output of sugar into the bloodstream during insulin action?
2. Are the muscles as well as the liver influenced by insulin?

Simultaneous analysis of the blood of the liver vein, the femoral artery and the femoral vein showed that the factors causing the fall of the blood sugar during insulin action are:

1. A diminished output of sugar by the liver into the blood stream.
2. A larger intake of sugar than normal by the muscle from the blood stream.

Insulin may cause a fall of blood sugar in three ways:

a. By its action on the liver (diminished output of sugar).

b. By its action on the muscle (increased intake of sugar).

c. By its combined action on both the liver and the muscle, the former showing diminished output and the latter simultaneously increased intake of sugar.

57 (2289)

Comparative study of the blood sugar concentration in the arterial and venous blood of diabetic patients during insulin action.

By CARL F. CORI, GEORGE W. PUCHER and BYRON D. BOWEN (by invitation).

[*From the State Institute for the Study of Malignant Disease, and The Buffalo General Hospital, Buffalo, N. Y.*]

The conclusions reached in the preceding abstract led to the study of the differences in blood sugar concentrations in arterial and venous blood of normal and diabetic subjects during the action of insulin.

The arterial blood was collected from the finger according to the technique of Foster.[1] Sugar was determined by the Hagedorn and Jensen method.[2]

The difference in the sugar concentration in arterial and venous blood of normal fasting individuals was found to be 5.5 mg. (average of 16 cases). For diabetic patients the difference in sugar content of the artery and vein was very variable ranging from 0-28 mg., the difference being in general higher than for normal persons.

During insulin action 6 out of the 7 diabetics examined showed a larger intake of sugar by the muscle. The highest observed difference was 35 mg. One case showed a diminished intake of sugar by the muscle during insulin action dropping to 0 three hours after the insulin injection. This indicates, as in the case of animals, that diabetic patients may react in several ways toward insulin. No conclusive data have as yet been obtained regarding the behaviour of the muscle of normal subjects during insulin action. In the three cases examined the muscle took up more sugar only during the early period of the insulin action.

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[1] Foster, *J. Biol. Chem.*, 1923, lv, 291.

[2] Hagedorn and Jensen, *Biochem. Z.*, 1923, cxxxv, 46.

58 (2290)

The effect of x-rays on the skin of vitally stained white mice.

By GERTY T. CORI (by invitation).

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

A unit of x-ray energy for the mouse has been established which could be compared with the human erythema dose. The amount of x-ray which caused total spontaneous epilation was called the epilation dose. It was found to be five to six times more than the human erythema dose. The effect of x-rays on the skin of mice stained with Trypan blue has been studied. It was found that the reaction of the radiated skin in stained and control mice is not markedly different, the sensitiveness of the stained mouse being slightly above that of the control mouse. There was, however, a marked difference between the normal and stained mouse in the time at which epilation began after the radiation. Different radiation times, 80, 70, 65, 60 and 50 minutes were applied. The epilation always occurred earlier in the stained than in the control mice.

59 (2291)

Variations in acetone excretion at different times during the day.

By ROGER S. HUBBARD and FLOYD R. WRIGHT.

[*From the Laboratories of the Clifton Springs Sanitarium, Clifton Springs, N. Y.*]

Determinations of the acetone bodies in urine were made every two hours during the day and on a single specimen collected at night in five experiments upon four subjects. In one experiment the diet was normal, in one it contained 1 and in the others from 1.1 to 1.3 molecules of ketogenic for each molecule of antiketogenic material. These figures were calculated from Woodyatt's[1] formula. In all cases the diets were planned to meet the estimated caloric needs of the subjects, and each patient had received the diet for several days before the experiment was carried out. The rate of acetone excretion in the normal subject and in the one who was receiving one molecule each of ketogenic and antiketogenic material was approximately constant, but in the others there were marked variations in that rate. The maximum rate occurred in the periods which began about two hours after the meals were eaten, and the minimum rate in the periods which immediately followed the ingestion of the meals. The average excretion at night was lower than in the daytime. These variations were considered as showing that the ingested carbohydrate was burned first, and that later mixtures richer in fat were burned.

If any patients for the whole twenty-four hour period had excreted acetone at the maximum rate found for any two hour period he would not have excreted the amount predicted on the assumption that one molecule of ketogenic is oxidized simultaneously with each molecule of antiketogenic material. The authors believe that such irregularities in excretion as are represented here account for the excretion of acetone which has been noted when diets relatively low in carbohydrates are fed to normal subjects; the fact that the excretion was very small in some of the specimens obtained in the period which followed immediately after the meals were eaten seems to show that variations at different times in the day are of more importance in causing such an excretion than are variations in the mixtures of foodstuffs oxidized simultaneously in different parts of the organism.

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[1] Woodyatt, R. T., *Arch. Int. Med.*, 1921, xxviii, 125.

60 (2292)

The relation between spontaneous activity and the ability to learn a simple maze.

By HOWARD S. LIDDELL (by invitation).

[*From the Department of Physiology, Medical College, Cornell University, Ithaca, N. Y.*]

In order to determine the effect of thyroidectomy on the neuro-muscular mechanism of sheep, methods were devised for the estimation of spontaneous activity and intelligence.[1] Activity was measured by a pedometer and intelligence was estimated by causing the animals to learn to escape from a labyrinth with a single cul de sac, the position of which could be reversed by a system of gates. In the type of problem represented by the labyrinth in which the solution depends upon vigorous random movements rather than visual discrimination one would be led to expect some definite relation to exist between the spontaneous activity of an animal and its proficiency in learning to escape from the maze. If one found that those sheep which were most active also made the best record in learning the labyrinth, it would be necessary to conclude that proficiency in learning the maze was an indirect way of estimating the animal's spontaneous activity and not primarily a measure of intelligence. The attempt was, therefore, made to determine for sheep the relation between activity and learning.

The experiments consisted in recording the spontaneous activity of a number of sheep in the flock and in comparing the total number of steps taken during a certain period with the records of the number of errors, steps, seconds, and trials obtained from causing the animals to learn the simple labyrinth and the labyrinth with the position of its cul de sac reversed. One hundred and fifty-two such comparisons were made between sheep which had learned the simple maze and one hundred comparisons between those which, after having learned the simple maze, had learned it again with the position of its cul de sac reversed. Activity was measured for periods ranging from four to thirty-seven days. An inspection of these results failed to discover any constant or close relation between learning and activity. It must be concluded, therefore, that even so simple a problem as the learning of this maze is a test, however crude, of intelligence rather than an indirect means of estimating activity. The expenses of this investigation were in part defrayed by a grant from the Sage Research Foundation.

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[1] Liddell, H. S., *Quart. J. Exp. Physiol.*, 1923, xiii, 191.

61 (2293)

The possible influence of fatigue on the reaction time of thyroidectomized sheep.

By HOWARD S. LIDDELL (by invitation).

[*From the Department of Physiology, Medical College, Cornell University, Ithaca, N. Y.*]

The time required by a sheep to escape from a simple maze which it has previously learned has been taken as a measure of its reaction to environmental change. It was found that a thyroidectomized sheep exhibits a greater reaction time than does its normal twin when it runs a simple maze in which the position of the cul de sac is reversed at every trial and four successive trials per day are given.[1] These preliminary results were obtained with a maze sixty feet in length. The greater time required by the cretin sheep was largely due to frequent pauses but also to its sluggish progression through the maze. It was suspected that muscular fatigue might be a factor of importance in accounting for the difference in reaction time between the normal and cretin sheep. In order to eliminate this possibility by reducing locomotion to a minimum a system of three gates was constructed whereby the same maze could be shortened until the distance from the starting box to feeding compartment was reduced to six feet.

The twin sheep, above referred to, were caused to learn the same alternation problem in this short maze that they had previously learned in the long maze. In this case, also, the cretin required a greater time than its normal twin to escape from the labyrinth. The difference between the normal and thyroidectomized animals was largely due to pauses made by the cretin in choosing which way to turn after leaving the starting box. The experiments with the short maze have, thus far, verified the hypothesis previously advanced, viz.: that the greater time required by the cretin to escape from the maze is due to the lethargic functioning of the neural mechanism involved in avoiding the cul de sac when its position is changed at every trial. The expenses of this investigation were in part defrayed by a grant from the Heckscher Research Foundation.

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[1] Liddell, H. S., *Proc. XI Int. Physiol. Congress*, Edinburgh, July, 1923.

ABSTRACT OF COMMUNICATIONS.

Minnesota Branch.

Thirteenth Meeting.

Minneapolis, Minnesota, November 15, 1923.

62 (2294)

Threshold densities of the electric current for inhibition and orientation of growth in Obelia.

By E. J. LUND.

[*From the Laboratory of General Physiology, University of Minnesota, Minneapolis, Minn.*]

If an electric current of proper density is passed lengthwise through an internode cut from the stem of the common hydroid *Obelia,* then the polyp on the end turned toward the cathode can be prevented from growing while a normal polyp will grow on the end turned toward the anode. The inhibition toward the cathode is reversible. The threshold density of electric current in the sea water for reversible inhibition on apical internodes is close to 66 microamperes per square millimeter of cross section. If an electric current of proper density is similarly passed through the internode at right angles to its long axis, then normal growth of a polyp occurs at each end, but the axis of symmetry in the regenerating tissue is deflected toward the anode. The magnitude of the angle of deflection depends in a certain definite quantitative manner upon the density of the electric current. The threshold density of the electric current in the sea water for a barely perceptible deflection of the growth axis is between 6 and 13 microamperes per square millimeter of cross section.

Intensities slightly above the threshold for orientation exert no apparent effects, other than orientation, upon the growth process.

The occurrence of a definite type of quite constant bioelectric potentials in the stem of *Obelia* has already been demonstrated.[1] Recently it has become possible to measure these electrical potentials of internal origin. The results show that the magnitude of these inherent potentials is many times greater than would be required for the orientation effects in the growth process and consequently demonstrate the possibility that the living cells of the tissue possess a mechanism which might be brought into action in the rôle of a mechanism for cell correlation in growing tissues. The detailed experimental facts at the basis of the above suggestion will be presented in full elsewhere.

63 (2295)

Electrodes for the measurement of small bioelectric potentials.

By E. J. LUND.

[*From the Laboratory of General Physiology, University of Minnesota, Minneapolis, Minn.*]

In connection with studies on certain types of small constant bioelectric potentials (about 0.5 millevolt) it has been impossible to use any of the electrodes in common use in physiological work.

The common Zn-$ZnSO_4$ non-polarizable type cannot be duplicated and marked variation in the E. M. F. with time occurs also. It was found that the E. M. F. of the more stable calomel electrode was susceptible to light and could not under the working conditions be maintained within a variation of 0.2 millevolt.

Cadmium amalgam $CaSO_4$ electrodes (saturated type of half Weston element) have not yielded the expected constancy under working conditions. The most stable electrode which has been tried so far, has been one made up with a three per cent pure lead amalgam and $PbCl_2$ in 0.05 to 0.01 molecular. The copper leads are dipped into copper amalgam instead of mercury. The drift in potential with time and at constant temperature was found to be sufficiently slow, and small enough to permit measurements of 1.0 to 0.05 millevolts with an error of about 0.01 millevolt under good conditions of experiment.

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[1] Lund, E. J., *J. Exp. Zool.*, 1922, xxxvi, 477.

64 (2296)

Iodine metabolism on normal diet in relation to prevention of goitre.

By J. F. McCLENDON and JOS. C. HATHAWAY.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

Since it would require 2,000 years to drink enough Lake Superior water to accumulate 20 mg. of iodine, and since the normal adult thyroid contains about this quantity and at least 50 per cent of persons drinking this or similar water escape goitre, there must be iodine in foods. We found that a man twenty-three years of age, living in a moderately goiterous region (Minneapolis), consumed 0.057 mg. of iodine in three days on a normal diet, and excreted 0.021 mg. of iodine during the same period, having retained 0.036 mg. of iodine in three days. At this rate, it would require about five years to accumulate 20 mg. of iodine.

The question arises why in a goiterous region some persons have goitre and some do not. From the following data it may be seen that persons eating much roughage and drinking much milk of herbivorous animals would be immune to goitre.

Food from Goiterous Region	mg. iodine per ton of dehydrated food
Wheat	1
Peeled and Cored apples	2.5
Oats (+ husk)	10
Skim milk	12
Spinach	18
String beans	29

The following table shows the cause for immunity in non-goiterous regions (figures denote mg. iodine per ton).

	Goiterous	Non-goiterous	
	Minnesota	Connecticut	Maine
Wheat	1	3.5	8
Oats (+ husk)	10	20	150

65 (2297)

Nutritive properties of taro root and poi and its possible relation to the preservation of teeth of the polynesians.

By J. F. McCLENDON, P. J. RILEY and AGNES EWELL.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

Dr. L. R. Sullivan of the American Museum of Natural History, and Dr. H. G. Chappel, examined historic and prehistoric Hawaiian skulls and found nearly all of them above forty years of age to show gingival caries of the teeth, even those buried before 1778 and hence before contact with the whites.

The principal food of the Hawaiians is poi made of taro root. The calorific value of dried poi is 4.17 kilogram calories per gram and hence about the same as starch. Dried poi contained 0.12 per cent phosphorus and 0.1 per cent calcium, and dried peeled taro root 0.12 per cent phosphorus and 0.06 per cent calcium. The difference in the calcium content may have been due to a different variety or sample of the root, or the fact that calcium of the water used in making the poi increased its value. The calcium and phosphorus values are low and comparable to the values for white flour and, like white flour, should be typical rachitic diets, unless containing a fair amount of antirachitic vitamine. White rats were fed a white flour diet containing 10 per cent casein and 1 per cent yeast, but two of these were fed one gram each of raw taro root per day. The taro root showed no antirachitic effect. On some days, however, not all of the taro root was eaten. In another experiment, one rat was fed one gram of boiled taro root per day which showed no antirachitic or anti-osteoporotic effect. Two controls were used. If any very large amounts of vitamine were present it should have been detected. It is therefore probable that the diet of the Hawaiians was no more antirachitic than that of ours in that poi is comparable to white bread. If the diet had anything to do with their caries it was the adult diet, since the children and adolescents had well developed, sound teeth. If it could be shown, statistically, that teeth were made worse by contact with the white man, it might be attributed to the reduced amount of sunlight reaching the skin in changing the native dress to European clothes. The association of Hawaiian caries with pyorrhea complicates the problem.

66 (2298)

The effect of hemorrhage on the blood after removal of the liver.

By F. H. SCOTT.

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*]

The principal factors determining the distribution of fluid between the blood and tissue spaces seems to be the filtering pressure of the blood in the capillaries tending to force fluid out, and the greater osmotic pressure of the colloids of the blood tending to pull fluid in. Normally, these two factors balance one another and the concentration remains fairly constant. As far as experiments go, the capillaries seem to be readily permeable to most crystalloids, especially salts.

The balance described above, however, is easily upset. Thus a rise of capillary pressure leads to a concentration of the corpuscles in the blood due to the filtering out of fluid (Scott[1]). Starling[2] showed the capillaries in the liver are the most permeable to colloid, the percentage of protein in liver lymph being practically that of the plasma. Histologists almost describe the capillaries in the liver as being devoid of a wall but they are not clear as to the origin of the lymphatics in that organ. With this very permeable membrane the influence of filtration should therefore be most marked in that organ, and this has been shown to be the case (Scott[1], Lamson and Roca[3]). The opposite conditions occur when capillary pressure is lowered; that is, the osmotic pressure of the colloid gains the upper hand, fluid is pulled into the vessels and the blood diluted (Scott[1]). The fluid which is pulled in is in all probability nearly pure salt solution, that is, it contains only a little colloid (Scott[4]). Seeing that the liver capillaries are so permeable to colloid, one would not expect the colloids of the blood to exercise much osmotic effect in that organ, but it was decided to test the point.

The easiest method of lowering capillary pressure is by hemorrhage. The great vaso-constriction produces a fall of capillary pressure and the blood becomes diluted. One cannot predict with certainty just how much dilution of the blood will occur in an animal for a given hemorrhage. The figures given below, however, will compare with the effects of hemorrhage in intact animals. Cats were used and anesthetized with chloroform followed by ether. A cannula was inserted into the carotid artery for taking samples of blood. After the cannula was inserted the abdomen was opened and the portal vein and hepatic artery identified just as they entered the liver. Clamps were then adjusted, but not tightened. The first sample of blood was then taken, the clamps then tightened and hemorrhage made. After a few minutes a second sample of blood was taken by allowing enough blood to escape to wash the cannula. This procedure, which not only excludes the liver but also the intestines, was followed in the first three experiments listed below. In the fourth experiment listed below, the intestines and liver were removed from the animal before the hemorrhage. In doing this, a considerable accidental hemorrhage occurred and it was not possible to make a large hemorrhage. However, the effect is very evident. The blood was kept from coagulating by means of oxalate and the hemoglobins compared as acid hematin in a Duboscq colorimeter, samples being prepared as described in previous papers.

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[1] Scott, F. H., *Am. J. Physiol*, 1917, xliv, 298.

[2] Starling, E. H., *J. Physiol.*, 1894, xvi, 224.

[3] Lamson, P., and Roca, J., *J. Pharm. and Exp. Ther.*, 1921, xvii, 481.

[4] Scott, F. H., *J. Physiol.* 1915, l, 128.

The hemoglobin in the first sample is considered 100.

Expt. I. Cat 2600 gm. hemorrhage 38 cc. hemoglobin red. to 87 in 3 min.
Expt. II. Cat 5090 gm. hemorrhage 80 cc. hemoglobin red. to 86 in 5 min.
Expt. III. Cat 2795 gm. hemorrhage 40 cc. hemoglobin red. to 93 in 2 min.
Expt. IV. Cat 3470 gm. hemorrhage 7 cc. hemoglobin red. from 75 to 70 in 2 min.
(The accidental hemorrhage caused the reduction from 100 to 75.)

The above results show that hemorrhage is about as effective in causing a dilution of the blood when the viscera are out of the circulation. This from the above considerations is what one would expect.

67 (2299)

Changes in the surface tension of broth produced by the growth of bacteria.

By W. P. LARSON and ROBERT DAVID EVANS

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

In work published from this laboratory, it has been shown that growth of bacteria is influenced by the surface tension of the culture medium. This fact has been recently emphasized further by the work of Ayers, Rupp and Johnson.[1]

The present study concerns the changes in the surface tension of broth produced by bacterial growth. Four samples of 1 per cent peptone broths were prepared in the usual way with some of the well known brands of peptones. They were inoculated with the *B. subtilis* and *B. coli,* respectively. The surface tensions were measured at 48 hour intervals by the drop weight method after clarifying the cultures by rapid centrifugation.

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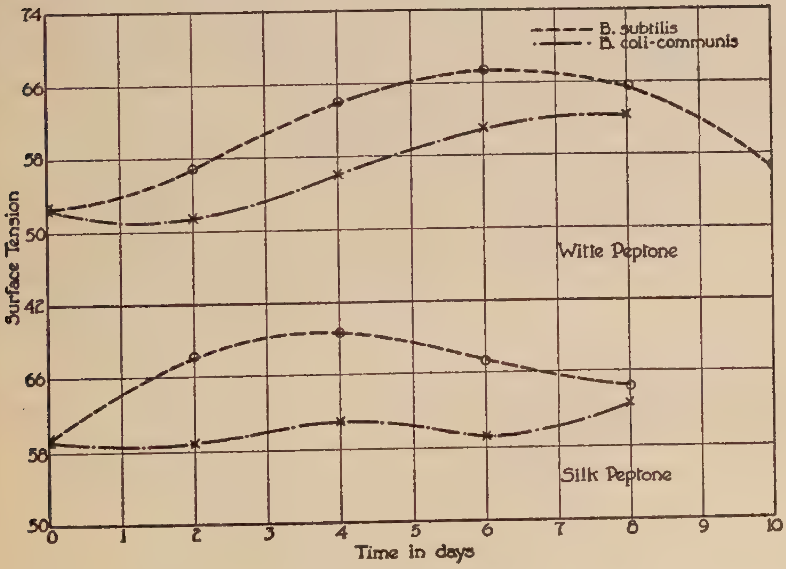
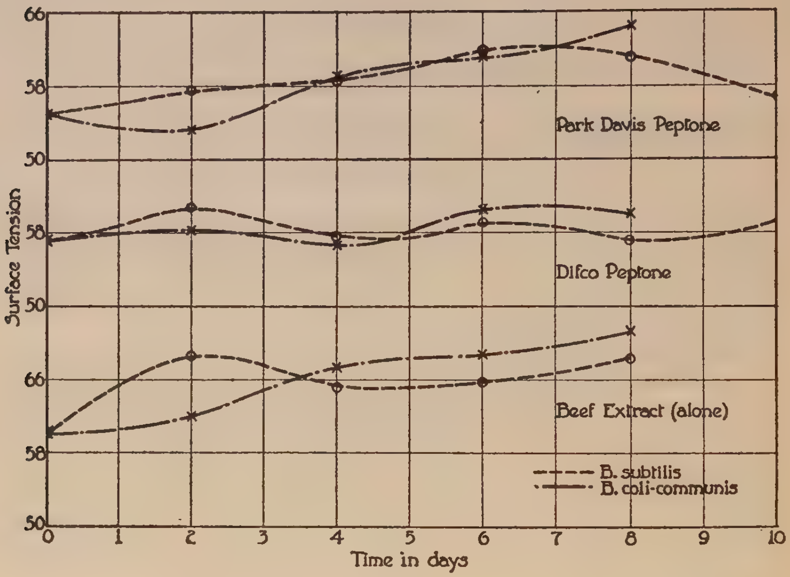
[1] Ayers, Rupp and Johnston, *J. Infec. Dis.*, 1923, xxxiii, 202.



The changes in surface tension are indicated by the accompanying graphs.



SCIENTIFIC PROCEEDINGS.

ABSTRACT OF COMMUNICATIONS.

One hundred and thirty-fifth meeting.

College of Physicians and Surgeons, Columbia University, December 19, 1923.

President Jackson in the chair.

68 (2300)

The uric acid content of the blood of the stellar sea-lion.

By NORRIS W. RAKESTRAW and ROBERT E. SWAIN.

[*From the Department of Chemistry, Leland Stanford Jr. University, Stanford University, Calif.*]

A year ago, in the course of a general investigation of the body fluids of the Stellar sea-lion, indigent to the coast of northern California, it was observed, quite accidentally, that the uric acid of the blood apparently undergoes a considerable increase on standing. A recent and more complete study of the same question has confirmed the earlier results completely. The uric acid content of the fresh blood of four animals was found to vary from a bare trace to two milligrams per hundred cubic centimeters, according to the method of determination used. After standing for eight days on ice the apparent uric acid content had increased to as high as seven milligrams per hundred cubic centimeters, and remained at approximately this level until the thirtieth day, when the experiments were discontinued. Even after eight hours of standing at room temperature the apparent uric acid content had increased considerably.

The determinations were made by three different methods, the direct colorimetric method of Benedict; the zinc precipitation method of Morris and Macleod; and the silver precipitation method of Folin and Wu. The three methods did not give concordant results for the same samples of blood, the amounts of uric acid indicated in each case being in the descending order in which the methods are listed above. It is significant, however, that the *difference* in the results from the three different methods remained practically constant, irrespective of the absolute uric acid level. In other words, the substance which accumulates in the blood on standing is precipitated by both zinc and silver. Whether or not these discrepancies in the results from the three methods are inherent in the methods themselves is of no concern in this present investigation. Actual uric acid—or at least a crystalline substance showing the murexid reaction—was isolated in small amounts from samples of the defibrinated blood.

The uric acid which accumulates on standing does not apparently result, however, from the simple hydrolysis of a combined form of uric acid (as Benedict has shown to be the case in beef blood), for acid hydrolysis had no appreciable effect upon the free uric acid content.

Using the technique of Davis, Newton and Benedict,[1] an effort was made to isolate, from fifteen liters of defibrinated blood, a possible uric acid-pentose compound, similar to the substance which these workers obtained from beef blood. No such compound could be isolated from the sea-lion blood. The precursors of the uric acid which accumulates in the two cases are thus entirely different.

The accumulation in the sea-lion blood is probably dependent in some way upon the corpuscles, since pericardial lymph—which is doubtless fairly representative of the blood plasma—does not exhibit an increase in uric acid on standing.

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[1] Davis, A. R., Newton, E. B., and Benedict, S. R., *J. Biol. Chem.*, 1922 liv, 595.

69 (2301)

The influence of sweat baths on the composition of the blood.

By H. C. GRAM (by invitation).*

[*From the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia, Pa.*]

The present series of observations was carried out to throw some light on the blood changes due to sweat baths (electrical bake) in various patients. The factors determined were the change in hemoglobin (with the initial value arbitrarily put at 100 per cent) the protein concentration of the serum, the osmotic pressure (freezing point depression) and the conductivity of the serum corrected for protein influence. The duration of the sweat bath was thirty minutes and no water was given. The blood was drawn from a cubital vein immediately before and after the bath. The results on eight patients—of which one was examined twice—were in brief as follows:

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*Robert M. Girvin Fellow in Research Medicine and Fellow of the Rockefeller Foundation.

Case	Diagnosis	Date	Time of Bleeding	Serum protein		Freezing point of Serum		Corrected NaCl equivalent of serum conduct		Percentile change in Hemoglobin*
				gm. per 100 cc.	percentile change	°C	percentile change	gm. per 100 cc.	percentile change	percentile change
M. C. B.	Chronic	May 3	Before bath	6.8	+13.2	0.560	+2.7	0.809		
1	Nephritis		After bath	7.7		0.575		0.827	+2.2	+2
A. G.	Hypertension	May 8	Before bath	7.1	+ 8.5	0.565	0	0.800		
2	Nephritis		After bath	7.7		0.565		0.807	+0.8	+5
M. Z.	Neuritis	May 24	Before bath	7.3	+ 8.2	0.560	+2.7	0.798		
3			After bath	7.9		0.575		0.807	+1.1	+3
K. K.	Nephritis	June 12	Before bath	7.5	+ 6.7	0.560	+1.8	0.802		
4			After bath	8.0		0.570		0.819	+2.1	+5
M. C. B.	Chronic	Sept. 18	Before bath	7.9	+ 2.5	no		0.836		
5=1	Nephritis		After bath	8.1		det'n		0.835	—0.1	+1
N. S.	Rheumatism	May 17	Before bath	7.9	+ 5.1	0.555	+0.9	0.793		
6			After bath	8.3		0.560		0.805	+1.5	+4
C. K.	Chronic	May 10	Before bath	8.0	+ 3.8	0.555	0	0.791		
7	Fibrositis		After bath	8.3		0.555		0.797	+0.8	+1
C. E.	Chronic	May 8	Before bath	8.3	+ 6.7	0.560	+1.8	0.797		
8	Nephritis		After bath	8.9		0.570		0.809	+1.5	+5
B. F.	Hypertension	May 15	Before bath	9.0	0	0.575	0	0.821		
9			After bath	9.0		0.575		0.824	+0.4	0
			Average		+6.1		+1.2		+1.1	+2.9

*All the absolute hemoglobin percentages on these patients lie between 90 and 110.

The conclusion may be drawn that the changes in the different constituents of the blood are not proportionate inasmuch as the protein concentration generally increases more than either the total electrolytes, the total dissolved substances or the hemoglobin. Concerning the relation between the hemoglobin and the serum protein change, it must be remembered that if the water-loss comes from the serum, which is roughly 50 per cent of the blood, then the increase in serum protein should be double the increase in hemoglobin; this is very nearly true in our average figures (6.1 and 2.9). It is also evident that the degree of concentration occurring as a result of the bake, if measured by the change in serum protein content, tends to be larger the lower the initial serum protein content. The fluid lost from the serum in excess of that gained from the tissues appears to be a salt solution of lower concentration than the serum.

70 (2302)

Studies on the chemistry of cod liver oil.*

I. The effect of hydrogenation upon the vitamine content.

By HARRY E. DUBIN and CASIMIR FUNK.

[*From the Biochemical Department of the H. A. Metz Research Laboratories, and the Biochemical Laboratory, College of Physicians and Surgeons, Columbia University, New York City.*]

A survey of the literature reveals comparatively little on the hydrogenation of cod liver oil. Drummond,[1] working with whale oil, hydrogenated at 250° C. for 4 to 6 hours, found that the hardened oil was entirely deficient in "fat-soluble A," which at that time was generally also regarded as the "antirachitic vitamine". Since then the bulk of evidence has tended to show that

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*This investigation was started in January, 1922, and is still being continued.

[1] Drummond, J. C., *Biochem. J.*, 1919, xiii, 81.

"fat-soluble A"—the antiophthalmic, growth-promoting vitamine—is not identical with the antirachitic vitamine.

Paal and Roth,[2] studying the catalytic action of colloidal metals of the platinum group, hydrogenated cod liver oil and butter. The process used by these investigators is a very mild one. As modified to suit our needs, it is carried out at about 55° C. for a period of about 36 hours. This is in sharp contrast to the drastic hydrogenation reported by Drummond.

Employing both the curative and preventive type of experiment the various fractions obtained by us, and about to be described, were tested on more than 150 rats. The diets used included a synthetic diet practically free from antirachitic vitamine, the McCollum diet No. 3143 and the Pappenheimer-Zucker diet D.

Fraction I.—This fraction, which possesses no vitamine activity, is the concentrated water reduction mother-liquor.

Fraction II.—The hydrogenated cod liver oil, obtained as a snow-white product, is odorless and tasteless, and melts at about 55° C. The yield is about 95 gm. from 100 gm. cod liver oil. When used in amounts corresponding to 0.100 gm. cod liver oil daily, it prevents and cures rickets. Smaller dosage was not tried.

Fraction III.—The residue left after extracting fraction II with alcohol. The yield is about 90 gm. from 95 gm. of fraction II. Fraction III is inactive in amounts corresponding to 0.250 gm. cod liver oil daily. Larger doses were not tried.

Fraction IV.—A creamy-white substance, almost odorless and tasteless, melting at about 30° C., extracted from fraction II by means of alcohol. The yield is about 4.5 gm. from 95 gm. fraction II. When given in amounts equivalent to 0.100 gm. cod liver oil daily, it prevents and cures rickets. Smaller dosage was not tried.

Fraction IVa.—This is the same as fraction IV, resembling it in appearance, but with the cholesterol removed. Yield is about 0.045 gm. from 4.5 gm. fraction IV. Administered in amounts corresponding to 0.250 gm. cod liver oil daily, it prevents and cures rickets. Smaller dosage was not tried.

Fraction C.—The cholesterol fraction proved to be inactive.

In the few cases in which the above active fractions were tested in attempts to cure ophthalmia, unsatisfactory results were obtained. However, we do not conclude that hydrogenation destroys the antiophthalmic vitamine, inasmuch as growth is a better index than ophthalmia, and our animals grew fairly well.

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[2] Paal, C., and Roth, K., *Ber. chem. Ges.*, 1908, xli, 2282; 1909, xlii, 1541.

In this connection, it is of interest to note that Goldblatt and Zilva,[3] using the preventive type of experiment with animals fed on the McCollum diet No. 3143, found that 33.7 mg. daily of a hardened cod liver oil protected against rickets, and that 1.8 mg. promoted growth. The original oil, before hardening, also promoted growth in daily doses of 1.8 mg. The antirachitic value of the original oil was not determined.

From the above, it appears that the vitamine potency of cod liver oil is not appreciably destroyed by a suitable hydrogenation process.

It is thus possible to prepare a potent concentrate which may be used as a starting point for further chemical fractionation, and as a source of vitamine possessing obvious advantages over cod liver oil. Further work along this line is in progress and detailed data will appear in an early publication.

71 (2303)

The occurrence of gastric lesions in rats, and their possible relation to dietary deficiency.

By ALWIN M. PAPPENHEIMER and LOUISE D. LARIMORE.

[*From the Department of Pathology of the College of Physicians and Surgeons, New York City.*]

In examining the gastro-intestinal tract of rats which were being used for other experiments our attention was directed to the frequent occurrence of lesions in the rumen or esophageal portion of the stomach. These lesions, in brief, consisted of an inflammatory edema of the mucosa and submucosa, with marked cellular reaction, and in most cases, localized ulceration. Associated with these inflammatory changes there was always present more or less epithelial hyperplasia. These lesions were situated by preference near the elevated ridge which divides the rumen from the glandular portion of the rat's stomach.

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[3] Goldblatt, H., and Zilva, S. S., *Lancet*, 1923, ii, 647.

A relation of the lesions to dietary factors is strongly suggested in the following table:

Diet*	No. of rats examined	No. of rats showing lesions	Percentage showing lesions
Stock	19	0	0
84	14	12	86
Modified 84 and 85	8	3	37.5
84 + C.L.O. (12-22 dps.)	7	2	28.5
—A	14	7	50
—A + C.L.O.	6	0	0
Full-diet alkaline	7	0	0
Full-diet acid	7	0	0
Full-diet neutral	4	0	0

It is seen from the above figures that none of the 43 rats on complete diets showed gastric lesions; whereas of 36 rats on variously deficient regimens lesions were present in 20, or approximately 55 per cent. It seems justifiable to infer that the occurrence of the lesions is in some way determined by the diet. We are not as yet able to define this relationship in terms of known deficiencies. The relative acidity or alkalinity of the diet, when complete in regard to its basic food constituents, appears to be without influence. The addition of cod liver oil to a diet deficient only in fat soluble vitamine afforded complete protection in the small series studied. The indications are, therefore, that the deficiency in fat soluble vitamine is an important factor, but further experiments are in progress.

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*Details as to the composition of the diets will be given in the final publication.

72 (2304)

Maintenance values for the proteins of milk, bread-and-milk, meat and soy bean curd in human nutrition.

By MARY SWARTZ ROSE, GRACE MacLEOD and BERTHA BISBEY.

[*From the Department of Nutrition, Teachers College, Columbia University, New York City.*]

Thomas (1909) endeavored to determine "biological values" for a number of foods, including milk, meat, and wheat flour, but none of his periods was long enough, nor were the nitrogen and energy intake uniform enough to determine maintenance values. Recently Martin and Robison have reported experiments more suitably planned, using milk and whole wheat bread, but their energy allowance seems above actual requirement, especially in the case of milk, yet their results show failure to attain nitrogen equilibrium on as much as 0.11 gm. of milk nitrogen per day, while their work on bread is at variance with that of Sherman and his co-workers, who found about 0.6 gm. of protein per kilo of body weight adequate for maintenance of nitrogen equilibrium over 12 and 15 day periods without increase of total energy intake above normal requirement when about 90 per cent of the protein was derived from a cereal grain. It seemed desirable to continue investigation of this matter.

A basal diet was devised which was eaten without difficulty, being fairly palatable and easily digested. It contained arrowroot starch, dextrimaltose, lactose, butter fat and apple. This never yielded more than three per cent of the total nitrogen of the diet, 97 to 98 per cent coming from the particular food under investigation. The fluid intake was uniform throughout, to guard against irregular flushing out of the nitrogen.

The subjects were two healthy women, each taking daily 40 calories and 0.077 to 0.08 gm. of nitrogen per kilo of body weight (or approximately 4 gm. per day), over 12 or 15 day periods preceded by a short preliminary period to adjust to the diet.

The results are summarized in the following table:

Subject	No. days	Source of Protein	N per kg body wt. gm.	N from protein studied per cent	N intake gm. per day	N output gm. per day	Balance gm.
T.Y.Y.	12	Soy bean	0.08	97.0	4.00	3.97	+0.03
B.B.	15	Meat	0.08	97.6	4.32	4.26	+0.06
B.B.	12	Milk	0.077	97.6	4.15	3.60	+0.55
B.B.	12	Milk and bread	0.077	50.0 48.0	4.12	3.72	+0.41

The relative efficiency of the four sources of protein used in the present experiments is best brought out by a comparison of the total protein storage by twelve day periods.

Food	Protein storage in 12 days (N × 6.25) gm.
Soy Bean	2.12
Meat	4.33
Milk	41.44
Bread-and-milk	30.37

If we assume a food to be efficient for maintenance when nitrogen equilibrium can be established on 0.08 gm. of nitrogen per kilo of body weight, the energy intake being adequate to maintain body weight, and assign this a value of 100 per cent, then the "biological" values for the foods under consideration might be stated in the following way, to express their relative value:

$$\frac{\text{Standard Requirement}}{\text{Actual Requirement}} \times 100, \text{ giving the results below.}$$

N intake	N for maintenance	Difference	Biological Value
gm. per kg. 0.08	gm. per kg. 0.08	gm.	100
Soy bean 0.08	0.079	+0.001	101
Meat 0.08	0.079	+0.001	101
Milk 0.077	0.066	+0.014	121
Milk and bread 0.077	0.069	+0.011	116

73 (2305)

Regarding the formation of ethereal sulfates.

By J. A. MULDOON, G. J. SHIPLE, and C. P. SHERWIN.

[*From the Chemical Research Laboratory of Fordham University, New York City.*]

Jaffé[1], also Baumann[2] found that, when brom-benzene is fed to a dog on a protein diet, the substance is excreted in combination with an acetylated cystine, *i. e.,* as brom-phenyl-mercapturic acid. We found that there was also a marked rise in the output of ethereal sulfates, amounting to from six to ten times the normal amount excreted.

We were unable to find any other animal, which excretes phenyl mercapturic acid under these conditions. Therefore we thought this might be a common intermediary product, in the formation of ethereal sulfates.

A pig was placed on a carbohydrate diet. It was first fed phenol, later *p*-chlor-phenol and lastly brom-benzene. With each of these substances, sodium sulfate was fed for a period of two days, then later, cystine, for the same length of time.

Inorganic sulfates have no effect on the ethereal sulfate formation. Cystine slightly increases the output of ethereal sulfate when phenol is fed but decreases it when *p*-chlor-phenol or brom-benzene is fed. This is probably due to the protective actions of cystine on tissue protein. Ethereal sulfates seem to chiefly come from endogenous catabolism. Brom-phenyl mercapturic acid when fed, seems to slightly increase the output of ethereal sulfates. However, this increase is insufficient to prove it to be an intermediary product of sulfur oxidation.

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[1] Jaffé, M., *Ber. chem. Ges.*, 1879, xii, 1092.

[2] Baumann, E., and Preusse, C., *Ber. chem. Ges.*, 1879, xii, 806.

74 (2306)

The absence of one kidney associated with hereditary abnormalities in the descendants of x-rayed mice.

By HALSEY J. BAGG.

[*From the Huntington Fund for Cancer Research, Memorial Hospital, and Cornell University Medical College, New York City.*]

Adult male and female mice were irradiated with comparatively light doses of unfiltered x-rays. They were mated and a few of their offspring in the second and third generations showed marked deformities of the eye. The eye abnormality was extensively inbred and after several generations all the individuals of many litters have shown the deformity in one or both eyes. Genetic tests have shown that the eye abnormality behaves as a Mendelian recessive.[1]

Club feet have been prominently associated with the eye defect. One or more limbs may be affected.

The young from such defective lines were killed *in utero,* and it was found that the structural defects of the eye were associated with arrests in development due apparently to a tendency to blood vascular extravasation in the region of the eye. In a few instances lesions were noted in the region of the foot, suggesting that a similar blood vascular disturbance and arrest in development may have brought about the club foot condition.

During the last few months the defective lines have become well established, and it has been possible to perform many autopsies on adult animals showing eye and foot defects.

Three hundred autopsies have been recorded and 50 animals (16 2/3 per cent) have shown practically complete absence of one kidney. In two instances very small remnants of renal tissue have been detected, which were not more than 1 mm. in diameter. Histological examination reveals apparently normal adult kidney tissue. One animal was blind in one eye and the other had apparently normal eyes. Two adult animals were found in each instance with a large cystic mass replacing one kidney. No normal kidney tissue was present. One of these animals was blind in both eyes and the other had apparently normal eyes. Each of the remaining 46 animals showed apparently complete absence of one kidney. One ureter in each case was missing, but both adrenals have been found in all animals. One animal killed at birth was found to have but one kidney.

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[1] Dr. C. C. Little was associated with the writer during the years 1920 to 1922, during which time the genetic tests were made. The results have been submitted for publication. See Little and Bagg, *J. Exp. Z.*, and Bagg and Little, *Am. J. Anat.*

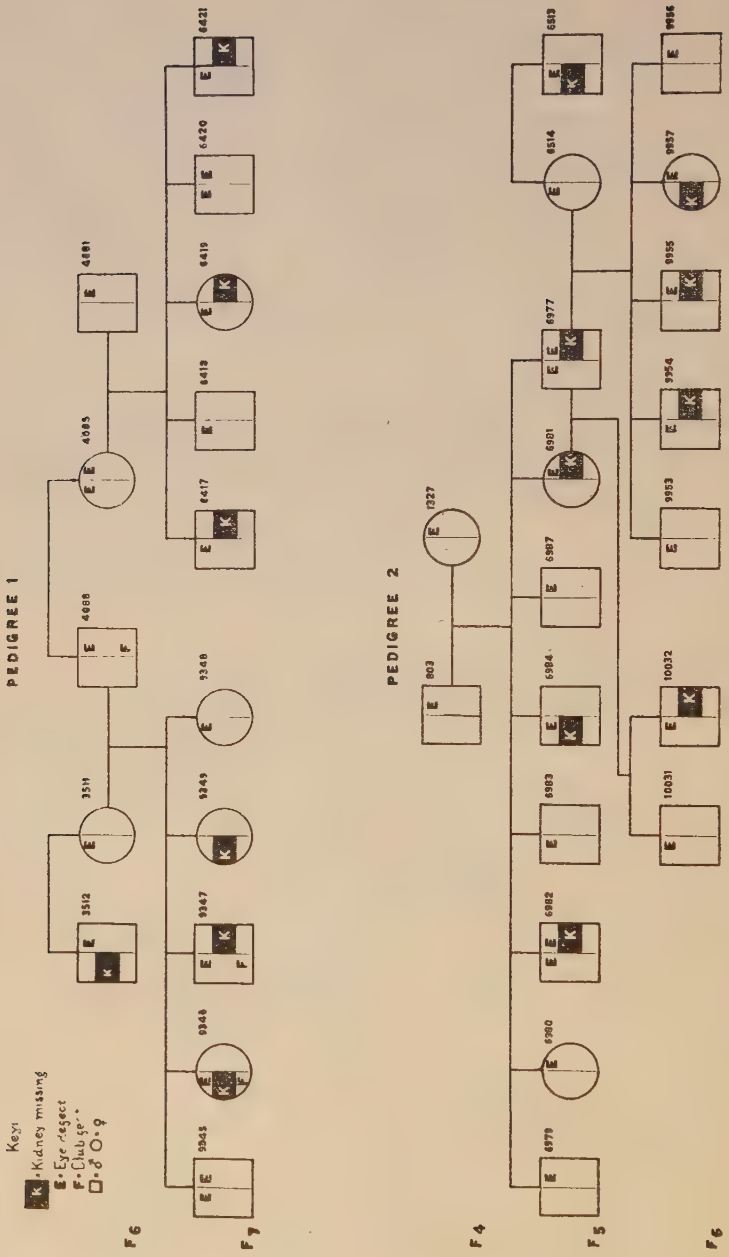
No. of animals with right kidney missing	25
No. of animals with left kidney missing	25
No. of animals with right eye abnormal and one kidney missing	14
No. of animals with left eye abnormal and one kidney missing	19
No. of animals with both eyes abnormal and one kidney missing	6
No. of animals with eyes normal and one kidney missing	11

Nineteen of the 33 animals with but one eye abnormal showed the eye and kidney defect on the same side of the body. Four of the six animals that were blind in both eyes had in each instance a missing right kidney and in the remaining two cases the left kidney was missing. Five of the eleven animals with normal eyes had a missing right kidney and six a missing left kidney. In three instances the association of defects was pronounced. The eye, club foot and kidney abnormalities were present in each animal.

Two examples of preliminary genetic results:

The entire seventh generation litter (Pedigree 1, Nos. 6417-6421 incl.) was killed at one time. Three animals were found each with a right kidney missing. At about the same time No. 3512 was killed and found to have a left kidney missing. As indicated in the chart the sib of the mother of the first litter mentioned was mated with the sib of 3512. Their offspring constitute litter Nos. 9345 to 9349 inclusive. Three of these five animals have in each case a missing kidney and in two instances the eye and foot abnormalities are also present.

The presence of the kidney abnormality has been only recently detected, so it is not known whether the ancestors of Nos. 803 and 1327 (Pedigree 2) carried the tendency for the condition or not. They probably did. The fifth generation litter of these two animals (Nos. 6979 to 6987 incl., 2 died, condition undetermined) showed four animals with one kidney missing, and two of these were also blind in both eyes. No. 6981 was mated with 6977. They each lacked one kidney. The offspring are shown in the sixth generation as Nos. 10031 and 10032, the lat-


PEDIGREE 1
PEDIGREE 2
Key:
K - Kidney missing
E - Eye defect
F - Club foot
□ - ♂ ○ - ♀
F 6
F 7
F 4
F 5
F 6
10031
10032
E
K
F

ter having but one kidney. No. 6977 was also mated with No. 6514 (a sib of 6513 having but one kidney). Their offspring constitute litter Nos. 9953 to 9957 inclusive. Three of the five animals have in each instance but one kidney.

The results so far obtained have shown that the kidney abnormality may be greatly increased in the experimental stock by breeding from animals heterozygous for this condition. Genetic experiments are now under way to determine the inheritance of the kidney abnormality by out-crossing with normal unrelated stock.

75 (2307)

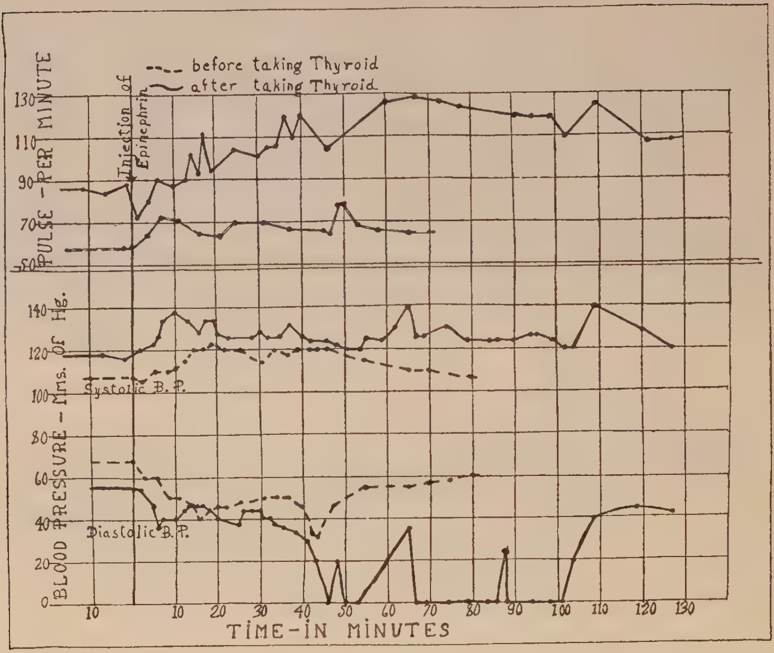
An unusual sensitizing action of thyroid substance on the effect of epinephrin in man.

By RUTH M. MORRIS, MARGARET S. WITTER, and SOMA WEISS.

[*From the Department of Medicine, Second (Cornell) Medical Division, Bellevue Hospital, and the Department of Pharmacology, Cornell University Medical College, New York City.*]

During a study on the behavior of the autonomic nervous system in man, the following observation was made:

A woman 27 years old, suffering from hypothyroidism received epinephrin, 0.015 mg. per kilogram of body weight (total 1.16 mg.) (tablets of Parke, Davis and Company) solution 1:1000, subcutaneously. The systolic and diastolic blood pressure and the pulse rate were recorded for 95 minutes following the injection. Readings were made at intervals averaging three minutes. The chart shows that there was only a slight rise (max. 12 mm.) in the systolic and a relatively marked fall (max. 34 mm.) in the diastolic blood pressure. The pulse rate showed a maximum increase of 22 beats per min. The patient felt comfortable during and following the test except for a slight tremor lasting a few minutes.



Following the injection of epinephrin the patient took an average of 0.8 gm. (14 gr.) of dessicated thyroid substance (Burroughs, Wellcome Tabloids) daily for seven weeks (47 gm. or 780 gr., total). During the last three weeks the patient experienced occasional tremor of the fingers, perspiration, a sensation of warmth and was excitable. She lost 11½ pounds in weight in the last four weeks of the thyroid administration.

The basal metabolism (Miss L.) was 87.5 per cent with respiratory quotient 0.803 before taking thyroid, and 109 per cent with respiratory quotient 0.769 following the administration of the substance.

After the thyroid substance had been taken, the epinephrin test described above, was repeated, with the same dose and the same sample of tablets. The result of the second test was rather surprising· As the chart shows, while the systolic blood pressure showed a marked rise (max. 22 mm.) which persisted longer than before, the diastolic blood pressure fell to zero. A typical Corrigan pulse developed and there was a distinctly visible capillary pulsation over the forehead. There was also a greater increase in the pulse rate (max. increase of 40 beats per minute);

and this increase was more persistent than in the first test. The heart beat was irregular with numerous extra-systoles. Corresponding to the marked change in the physical signs, there was also a marked disturbance in the subjective sensations of the patient following the administration of the thyroid substance. She developed marked trembling of the back, alternating with choreiform movements of the shoulders. These movements occasionally attained the severity of mild convulsions, which could be suppressed by the patient only by the utmost exercise of the will. These disturbances disappeared with the return of the blood pressure to normal, while the heart rate returned to normal later.

This clinical experiment suggests that large doses of thyroid substance caused sensitization toward the effects of epinephrin in such a way that, while the systolic blood pressure showed a greater and more persistent rise after the administration of thyroid than before, due to a constriction of the peripheral arteries and arterioles, the diastolic blood pressure fell to zero with the development of a capillary pulse, due, probably, to a dilation of the capillary system.

One may conclude, therefore, that following the administration of these large doses of thyroid substance, the dilator action of epinephrin on the capillary system was apparently antagonistic to its constrictor action on the peripheral arteries and arterioles.

76 (2308)

Intracardiac irradiation of the valves from radium emanation, employing a two-stage operative technique.

By P. CORYLLOS, D. J. EDWARDS, and H. J. BAGG.

[*From the Physiological Laboratory, and the Memorial Hospital, Cornell University Medical College, New York City.*]

In mitral stenosis there is indicated, on theoretical grounds, some form of intervention designed to dilate the narrowed orifice and thereby relieve the functional embarrassment of the heart. In advanced cases such relief must come through an intracardial approach. The operative dangers of such a course are necessarily great, but in hopeless cases of stenosis the chances of final success through opening-up of the constricted orifice, with the resulting advantages, may well outweigh the dangers. A single attempt in this direction has already been reported by Dr. Cutler, of Boston.

An important characteristic of mitral stenosis from the standpoint of its ultimate relief is the slow progressive nature of the lesion as it normally occurs. This makes it impossible to predict whether surgical dilation will effect a permanent relief or will only alleviate the condition temporarily, after which there will occur a reformation of the old stenotic ring. The latter possibility makes it irrational, in the present state of our knowledge, to expose a patient to the dangers of this surgical operation.

In view of these considerations we have been attempting in the past year to produce in dogs a real stenosis characterized by scar formation on valves and ring and presenting a progressive nature, as contrasted with the many previous attempts in this direction which have, for the most part, been in the nature of mechanical ligations of the orifice. With some reliable method of provoking a valvular lesion of this kind, it is our aim, then, to observe more fully the course of events following surgical intervention.

The work to date has shown that valvular disease induced by cauterization of the valves with sodium or potassium hydrate or by mechanical injury of these structures is impracticable. On the other hand, the use of strong doses of radium emanation (200 to 400 mc.) applied directly to the A-V valves and introduced in a special applicator through the appendix, has given promising results. In some hearts thus treated macroscopical evidences were shown of marked congestion and edema of the entire valve, together with congestion at the valve base and patchy thickening at the borders of the other leaflets.

Another aspect of the work presenting features of interest is the operative procedure. In the early experiments the mortality was high, due to shock, double pneumothorax, empyema, and pneumonia. In order to overcome many of these difficulties we worked out a very successful two-stage operation. In the first stage two or three ribs are resected in the precordial region. The parietal pleura thus exposed, but unopened, is treated with carbolic acid. This provokes some adhesions which serve in a measure to prevent lung collapse when the second stage of the operation is carried out two or three weeks later for the purpose of irradiating the valves.

77 (2309)

Viability of the intestine.

By H. B. EISBERG.

[*From the Department of Experimental Surgery, University and Bellevue Hospital Medical College, New York City.*]

The margin of safety has not been definitely determined concerning circulatory changes in a segment of intestine as the result of injury, thrombosis, embolism or mechanical intestinal obstruction. The work of Rydigier[1] closely followed by that of Madelung[2] showed that it was impossible to establish any definite rule as to which vessels of the mesentery of the small intestine could be ligated without danger of gangrene. The experiments of Mall and Welch[3] brought out the fact that necrosis occurred if much more than 5 cm. of intestine were separated from its blood vessels.

This report is based upon the observations in twenty-four dogs. The circulation to an intestinal segment in each experiment was interfered with by ligation of various vessels in different portions of the intestine. The results obtained were as follows:

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[1] Rydigier, L., *Berl. klin. Woch.*, 1881, xviii, 593; *und Deut. Z. Chir.*, 1884, xxi, 546.

[2] Madelung, O., *Arch. klin. Chir.*, 1882, xxvii, 227.

[3] Mall and Welch, *Albutt's System of Medicine*, 1902, vi, 239.

No. of Experiment	Duration of life	Location and Vessels Ligated	Pathology†
		Duodenum	
54*	11 days	5 contiguous terminals	Deep congestion central portion—return of circulation to ends of segment.
212*	31 days	2 contiguous terminals	Circulation returned to involved segment.
221*	58 days	3 contiguous terminals	Circulation returned to involved segment.
		Jejunum and Ileum	
8*	13 days	Cecal branch ileo-colic	Circulation returned to involved segment.
4*	13 days	1 first arcade	Circulation returned to involved segment.
18-A*	21 days	2 alternating second arcade	Circulation returned to involved segment.
20*	21 days	3 contiguous second arcade	Circulation returned to involved segment.
1*	13 days	3 contiguous terminals	Circulation returned to involved segment.
11*	10 days	3 alternating terminals	Circulation returned to involved segment.
6	24 hrs.	1 intestinal	Segment gangrenous, no perforation.
10	24 hrs.	1 intestinal	Segment gangrenous, no perforation.
18	36 hrs.	1 intestinal	Segment gangrenous, perforated.
56	10 days	3 contiguous first arcade	Segment gangrenous, no perforation.
215	84 hrs.	8 contiguous terminals	Segment gangrenous, perforated.
		Colon	
55*	6 mos.	3 alternating terminals	Circulation returned to involved segment.
223	60 hrs.	Middle colic	Segment gangrenous, perforated.
224	204 hrs.	5 contiguous terminals	Segment gangrenous, no perforation.
225	108 hrs.	5 contiguous terminals	Segment gangrenous, perforated.

From the data herein presented it appears that the circulation returns to a segment of gut when three contiguous vessels are tied, or when the cecal branch of the ileo-colic is tied, or after other experimental ligations, as represented in table. The circulation, however, does not return when the main artery is ligated, or more than one vessel of the first arcade, or more than four contiguous terminal arteries.

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* Euthanasia.

†Adhesions in all experiments.

Variables 16, 17-A, 19, 214, 226, 227, not included in this series, owing to twisting of segment producing a mechanical obstruction and peritonitis not the result of the experimental pathology.

78 (2310)

The role of the nucleus in the locomotion of an entozoic ameba.

By ELERY R. BECKER (by invitation).

[*From the Department of Biology, Princeton University, Princeton, N. J.*]

There is a large ameba, oval or circular in cross section, entozoic in the rectum of the bull-frog tadpole, which averages well over 100 μ in length, and from one-fifth to one-fourth as wide as long. It is strikingly peculiar in that it possesses an axial stream which flows swiftly and uniformly toward the anterior end, from which it is reflected about itself as a hollow cylinder of protoplasm either flowing posteriorly or remaining stationary with respect to particles on the substratum, depending on whether the ameba as a whole is "marking time" or moving forward. The large conspicuous nucleus always lies in a crescentic zone of clear ectoplasm at the extreme anterior end of the ameba, so that its posterior surface appears to be contiguous with the endoplasm.

That the nucleus plays a definite rôle in directing protoplasmic streaming, and thus possesses potentialities which influence strongly the shape of the ameba and the direction and manner of its movements is suggested by the following observations and experiments.

1. It can be observed that the nucleus oscillates from side to side in the anterior end of the ameba (though not in one plane as it appears to the observer through the low powers of the microscope). The endoplasm flows into the portion just previously occupied by the nucleus, the nucleus moving (presumably being forced) to a new position, into which the endoplasm immediately follows it. The process is steady and continuous. Thus the nucleus and anterior tip of the ameba both describe spiral paths.

2. Amebas which have been cut in two transversely, so that the anterior portion containing the nucleus presented about one-fifth the area of the entire ameba, show that the part containing the nucleus is able to resume normal shape and movements. The posterior portion always rounds up somewhat, undergoes some

violent and uncoordinated streaming movements, extends and retracts numerous pseudopods, without accomplishing any appreciable change of position or resuming the normal method of endoplasmic streaming or locomotion. It eventually rounds up completely, and streaming ceases, while the anterior portion may still be behaving quite normally.

3. If the nucleus and a negligible amount of ectoplasm be excised, the enucleated ameba behaves very much like the enucleate portion above. This eliminates the possibility that differentiation of the anterior cytoplasm is the factor which distinguishes its behavior from that of the posterior end.

4. If the nucleus be dislocated by pressure on the anterior end, it commences to travel posteriorly in the peripheral stream. After a moment the streaming is arrested. Then suddenly the ectoplasm bulges at the point where the nucleus has stopped, the endoplasm flows into the new pseudopod, and the ameba with the nucleus in its tip, travels away in the direction of the new pseudopod.

The nucleus seems in some way to play a rôle in directing endoplasmic streaming, presumably by its ability to slightly liquefy the ectoplasmic gel, lowering its resistance at certain points to the force of the endoplasmic stream.

79 (2311)

The synergistic toxicity of local and general anesthetics.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

The author has had occasion to determine the lethal dosages of cocain and alypin for cats by intravenous injection. Solutions of the drugs, generally 1 to 1000, were injected into the femoral vein at regular intervals and the lethal dose of each drug per kilo weight of cat was determined. It was found that the toxicity of both cocain and alypin was very much increased or potentiated when the animals were kept under complete general anesthesia. Thus it was found that whereas the lethal dose of cocain per kilo weight of cat when injected intravenously under very light anesthesia was from 30 to 40 mg., the fatal dose for cats under complete general anesthesia was about 25 mg. per kilo weight and under deep ether anesthesia it was as low as 15 mg. per kilo weight. In the case of alypin it was found that when injected in cats practically under no ether anesthesia, the lethal dose varied from 30 to 24 mg. per kilo weight; under complete but light ether anesthesia the lethal dose was from 18 to 24 mg. per kilo weight, while under deep anesthesia the fatal dose was as low as 6 to 7 mg. Similar toxic synergism in cocain and alypin on the one hand and general chlorbutanol anesthesia was also noted. The above experiments were performed several years ago but have been repeated recently with the same results and are deemed to be worthy of mention in connection with the present renewal of interest in the toxicity of local anesthetics. In the experience of the author a combination of local and general anesthetics is dangerous and this fact should be borne in mind by surgeons who may desire to switch from a local anesthetic to a general one.

80 (2312)

Note on the weight of the adrenals in crosses between the albino and the wild Norway rat (Mus norvegicus.)

By JOHN C. DONALDSON (by invitation).

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When the weight of the adrenal glands is compared with either the body weight or the body length, the wild pigmented rat is found to have much heavier glands than the domesticated albino. The females in both groups have heavier glands than the males, but the difference in weight between the glands of the two sexes is less in the wild gray form.[1]

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[1] Hatai, S., *Anat. Record,* 1914, viii, 511.

The following experiments were undertaken to determine the weight of the adrenals in descendants from albino by Norway ancestors. It was appreciated that these observations must necessarily be made under the conditions of domestication and that the effect of domestication must be considered in the interpretation of the results. Under these limitations the purpose was to observe in what way the weight of the adrenals was related to pigmentation.

A wild gray male was crossed with a stock albino female and the offspring used for this work. The weights of the adrenals in the original pair are, unfortunately, not available. The values found in the other wild gray rats captured in the same vicinity are, however, close to those given by Hatai.[1] In our colony the average values for the female albino adrenals vary less than one per cent from that given in Table 71, "The Rat".[2] One hundred and two animals were examined, covering four generations. Twenty-two of these, however, had pus in the ears or showed gross pathological changes in the viscera, usually the lungs. Since disease seems to influence the size of the adrenal glands, these animals were excluded and the eighty remaining animals were used in the computation.

The results are shown in Tables I and II. The body weights at the time of killing varied from 64 to 368 gm. in the males and 97 to 342 gm. in the females. The method used for comparing the different groups has been described.[3] The adrenal glands of the experimental animal were weighed. The expected weight of the glands of an albino rat, having the same body weight and sex as that of the experimental animal was then found from Table 71 in "The Rat". The observed weight was compared with the expected weight and the difference in the observed weight expressed in per cent of the expected weight. The individual percentage values of each group were then averaged. The values in Table I, column 3, were obtained in this way. If body length instead of body weight is used as a basis of comparison, the results are similar.

While the variability is great and the number of observations small, it is evident in both sexes that the pigmented groups have distinctly heavier adrenals. The F_1 generation shows a sharp reduction in the weight of the adrenal from that found in the wild form. This reduction is greater than that following domestication of wild grays even for several generations.[4] In the F_1 generation the excess in weight of female glands over those of the male is close to that found in the wild form. In subsequent generations this excess is reduced so that there is less difference between the weight of the glands in the two sexes than is found even in the wild gray rats. The relation between the groups of experimental animals is more clearly shown by Table II. The average weight of the adrenal glands for each sex of the albinos in the F_2-F_4 generations has been taken as 100 and the relations of the other groups expressed as percentage of this value.

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[2] Donaldson, H. H., *The Rat*, Data and Reference Tables, p. 133-138, Philadelphia, 1915.

[3] *The Rat*, pages 3-5.

The evidence shows that, body weight for body weight, the albinos have lighter adrenal glands than do pigmented animals of the same ancestry and raised under the same conditions. This difference is much less than that between the wild gray and domesticated albino forms. The experiments were carried on, however, under the conditions of domestication, which tend to lower the weight of the adrenals in the Norway, and the effect of domestication is thus added to that of the albino in reducing the weight of the adrenals in the pigmented rats.

TABLE I.

Group	No. of cases.	Av. adrenal wgt. expressed as a percentage above the expected wgt.	Probable error of the mean.
Males:			
Albino F_2-F_4	16	26.6	±3.9
Pigmented F_2-F_4	20	38.6	±4.0
F_1 generation (pigmented)	10	31.2	±1.4
Wild gray (Hatai)		114.0	
Females:			
Albino F_2-F_4	12	3.18	±2.2
Pigmented F_2-F_4	15	11.10	±3.1
F_1 generation (pigmented)	7	17.6	±2.7
Wild gray (Hatai)		91.0	

The expected weight is that given for each sex in Table 71, "The Rat," 1911.

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[4] At the Wistar Institute the reduction after three generations in captivity did not exceed 30 per cent in either sex. (Personal communication from H. H. Donaldson.)

TABLE II.

The average weight of the adrenals in the pigmented groups expressed as a percentage of the average weight of the glands as found for the experimental albinos.

Group	Male	Female
Albino F_2-F_4	100	100
Pigmented F_2-F_4	109.3	107.6
F_1 generation (pigmented)	103.1	113.9

81 (2313)

Parathyroid extirpation in the cat.

By J. S. NICHOLAS and W. W. SWINGLE.

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A survey of the literature dealing with experimental parathyroidectomy in the cat shows amazing discrepancies. Some results are so clear cut that there can be no question as to the conclusions drawn from them while other experiments give quite conflicting data. In certain forms, *viz.*, the dog, there are uniform results after extirpation of these glands and these have led to the conclusion that in this form a complete parathyroid extirpation is fatal.

In order to clear up some of these discrepancies before proceeding with physiological studies which are now under way upon the cat it was deemed advisable to complete a large number of parathyroid extirpations in order that a consistent resurvey of the problem could be obtained. More than fifty parathyroidectomies have been performed at the Osborn Laboratory. These cases have been carefully studied and supplemented with anatomical observations.

A microscopic examination of the glands was made at the time of operation. The glands were placed in Ringer's solution and the parathyroids dissected away from the thyroid under the high power of a binocular microscope. A small vascular rete is associated with the parathyroids and assists in their rapid identification.

In the cat the typical number of parathyroids (4) is not invariably present and the glands, especially the internals, vary considerably in size and position. The internal parathyroid may occur anywhere upon the dorsal surface of the thyroid or so deeply imbedded in its tissue that it can be found only in section. It may be absent on one side. The external parathyroids were more constant in their location but vary in size and in number. In several cases the gland was represented by several minute masses. Accessory glands were present in 35 per cent of the cats and these were most frequently found in the cervical lobe of the thymus.

In our first experiments, the operation was considered as complete when the four glands were located and identified. The animal was placed under observation and if tetany did not develop within four days, a second operation was undertaken in which the entire neck region was explored for parathyroid tissue. All tissues removed were carefully examined for parathyroid tissue. In the course of the second operation the cervical lobes of the thymus were removed and occasionally the operation was extended into the superior mediastinum. Accessory glands were found in the cervical thymus in so many cases that the original field of operation was enlarged so as to include all of this tissue.

In the large majority of cases, death from tetany ensued after this operation. Careful autopsies supplemented with the study of sections failed to reveal any parathyroid tissue remaining. In contrast to this, whenever an animal survived and showed no signs of tetany, parathyroid tissue was found at autopsy.

Typical tetany symptoms always occurred after complete extirpation of the parathyroids. The symptoms are of two types, a violent type in which the body is subject to tremors and convulsive attacks and a depression type in which there are no convulsive attacks. Either type is characteristic after parathyroid extirpation. A survey of our fifty cases showed that the violent type of tetany predominates as the cause of death.

82 (2314)

The fate of injected protein in an animal immunized against it.

By EUGENE L. OPIE.

[*From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa., and the Department of Pathology, Washington University, St. Louis, Mo.*]

Proteins have been selected as antigens capable of reproducing the reactions of immunity and the attempt has been made to determine if immunization by repeated injection modifies their absorption. In the normal rabbit proteins such as horse serum and egg albumin are absorbed with no conspicuous inflammatory reaction, whereas in the rabbit immunized by six or seven injections of the antigen at intervals of five days a subsequent injection produces an intense inflammatory reaction associated with necrosis at the site of introduction of the antigen (Arthus phenomenon). A former study[1] has shown that the injected horse serum, egg albumin or crystalline egg albumin introduced into an immunized animal fails to enter the blood in quantities demonstrable by the precipitin reaction, whereas, in the normal animal the antigen enters the blood and is demonstrable in considerable concentration. The present experiments were undertaken to determine the fate of the antigen at the site of injection. The first experiments were made with crystalline egg albumin. The presence of the protein in an extract of tissue removed from the site of injection was determined by the precipitin reaction and its concentration in a weighed amount of tissue determined by comparison with precipitin reactions obtained when the precipitating serum used in the tests was mixed with known concentrations of the antigen. Concentration of antigen at the site of injection in normal and immune animals at varying intervals after injection of 0.5 cc. of a 5 per cent solution of crystalline egg albumin is shown in the following table:

	Normal Animal	Immune Animal
1 day	1/200	1/20
2 days	1/500	1/50
3 days	0	1/250
4 days	....	1/750
5 days	....	0

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[1] Opie, E. L., *J. Immunol.*, 1923, viii, 55.

In the immune animal antigen was still demonstrable at the end of five days in the necrotic skin at the site of injection (Arthus phenomenon) but was absent in the surrounding indurated tissue as indicated in the table.

The foregoing figures show that a readily diffusible antigen such as crystalline egg albumin in an immune animal is fixed at the site of injection and for this reason fails to enter the circulating blood.

Similar experiments performed with horse serum showed that it had disappeared from the site of injection both in the normal and in the immune animal at the end of three days, but the evidence that the immune animal holds the protein at the site of injection is readily obtained by estimating the quantity of protein still present at the end of twenty-four hours.

In one experiment twenty-four hours after intradermic injection of 0.5 cc. of horse serum into a normal animal the amount obtainable at the site of injection was 0.17 cc. and the antigen was present in considerable quantity in the blood serum. After the same injection into an immune animal 0.449 cc. was recovered from the site of injection and none was found in the blood serum.

The experiments indicate that the immune animal differs from the normal in its ability to fix the antigen at the site of injection. It is probable that this fixation is brought about by specific precipitation. Associated with this precipitation there is an acute inflammatory reaction (Arthus phenomenon) and the precipitate formed by antigen and precipitate which is strongly chemotactic is probably destroyed by leucocytic digestion.

83 (2315)

The ovarian cystic fluid with special reference to its effect upon the reactions of the genital tract.*

By G. N. PAPANICOLAOU and N. F. BLAU (by invitation).

[*From the Cornell University Medical College, New York City.*]

Recent studies[1] on the morphology of the ovarian cysts tend to prove that their structures are, as a rule, not of a follicular origin, but result in most cases from the overgrowth of the embryological remains of the Wolffian duct system, part of which persists in a rudimentary form within the ovaries of mammals.

Viewed from this standpoint, ovarian cysts should not be considered solely as pathological structures. There is a normal element in their existence, the study of which might be of some interest both morphologically and physiologically.

Considerable work has been done on the morphology of the cystic structures but knowledge of the chemical nature and the physiological properties of the fluid contained within the cysts is very fragmentary.

With a hope that such a study might contribute something to a better understanding of the ovarian functions as a whole, we have undertaken an investigation of this problem, the first report of which is given in this preliminary note.

We have studied crude and barkefelded material taken from cysts of human ovaries as well as of ovaries of cows and pigs. Many of the human cysts were of parovarian type, thus excluding the possibility of a follicular origin. In some cases we have tested the fluid of cystic *corpora lutea*. Later we have investigated the effects produced by various constituents of the cystic fluid. Guinea pigs have been used as test animals.

The results obtained so far indicate that the cystic fluid has a decided physiological action and produces rather typical and characteristic effects upon the female genital tract.

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* This investigation has been conducted with the aid of The Committee for Research on Sex Problems of the National Research Council.

[1] Papanicolaou, G. N., and Stockard, C. R., Morphology of cystic growths in the ovary and uterus of the guinea pig, PROC. SOC. EXP. BIOL. *and* MED., 1922, xix, 401.

These effects may be summarized as follows: There is first a congestive reaction, which begins shortly after the initial injection and involves the entire genital tract from the ovary down to the uterus and the vagina. This congestion is followed by an increased function of the uterine glands, and by hypertrophic activity, giving a comparable picture of the changes occurring normally during the proestrum in the mammalian genital organs.

This increase in blood supply and function tends to shorten the diœstrum in normal animals, *i. e.,* the time interval between two oestruses, thus indicating a stimulating effect upon the genital mechanism.

In ovariectomized animals a similar proestrous condition is produced, which as soon as the effect of the injected fluid is past, ends with the destruction of the hypertrophic material in a way, which imitates some of the changes occurring during oestrum and metoestrum. This fact tends to support the view that the catabolic changes characterizing the oestrum in mammals are the result of a decrease in hypertrophic activity of earlier stages, resulting in the final destruction of the over congested structure.

The oestrous mechanism, when properly analyzed, may not be so complicated. An increased blood supply due to congestion may cause an increased function and a more or less hypertrophic condition, which later returns to the normal level.

For this reason it is unsafe to decide definitely on the specificity of all the oestrous like changes produced by the cystic fluid, since many of these effects can be explained as the indirect result of the original congestive condition. We also know that many factors may produce congestion in certain organs, without being of a specific nature. On the other hand we do not entirely exclude the possibility of a specific action.

Different constituents of cystic fluid, *e. g.,* those containing proteinic fractions, lipoid fractions, etc., seem to act in somewhat different ways. The reactions described above apply mainly to the lipoid extracts. Thus the possibility of attributing these effects to purely proteinic reactions may be entirely excluded.

It seems to us that a thorough study of the different fractions and their effects upon other important organs than the genital tract should be made before the question of specificity can be decided. The same must be said regarding the many other extracts reported as affecting specifically the genital mechanism. Much of the work on this subject has been based on incomplete studies, and an insufficient knowledge of the genital functions, and as such it cannot be considered as decidedly conclusive.

84 (2316)

The mechanism of feeding in the oyster.

By THURLOW C. NELSON.

[*From the Department of Zoology, Rutgers College, New Brunswick, N. J.*]

The food of the oyster consists of minute plants, plant detritus, and animals filtered from the water passing through the gills. In turbid waters much sand and other inorganic matter is accumulated along with the food particles. Bivalve molluscs which live in muddy waters possess highly complicated systems of ciliary tracts with the aid of which some separation of food from dirt does occur.[1] In the oyster this mechanism consists partly in the gills, but mainly in the palps. Water borne particles on striking the gills are entangled in mucus and carried by the cilia of the gill epithelium: (a) ventrad to the groove formed by the ventral margins of the gill lamellae; or, (b) dorsad to the dorsal groove which passes along the bases of the gill filaments.

In these grooves the mucus covered particles are whipped into slime strings which are then carried anteriorly to the posterior margins of the palps. At this point the material may either pass between the deeply grooved and heavily ciliated faces of the palps, and thence toward the mouth, or failing this is pushed off onto the mantle ventrad the edges of the palps. At intervals the material accumulated here is expelled from the mantle chamber by water forced out by quick contractions of the adductor muscle.

When relatively large masses of collected particles are brought to the palps most of these are rejected and pass off onto the mantle as Kellogg[2] has shown. A larger proportion of the material brought to the palps by the dorsal groove is accepted than that which arrives via the ventral groove. Observation shows that the larger particles, chiefly sand grains, are consigned mainly to the ventral groove. In their passage over the gills they evoke a relatively copious secretion of mucus which renders them bulky and consequently less likely to enter between the palps. The majority of food particles, which stimulate a somewhat less secretion of slime while passing over the gills, enter between the palps and are passed toward the mouth. The separation is by no means perfect as some food materials are rejected and much dirt and sand may at times be taken into the stomach.

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[1] Nelson, T. C., *J. Morph.*, 1918, xxxi, 53.

As the slime strings are passed between the opposed faces of each pair of palps still further rejection of materials is effected. This is accomplished partly as Allen[3] has shown through reflex erection of the ridges of the palps which brings into play groups of cilia which beat away from the mouth. Further separation is accomplished through the medium of the grooves between the ridges and the short transverse loops in these grooves which occur progressively more abundantly from about the middle of the palp toward the mouth. Small particles picked out of the slime strings by the cilia, pass down into the grooves, the cilia of which, in the anterior part of the palp, beat dorsad. Particles carried dorsad by these ciliary currents, when they encounter a transverse loop of the groove, pass across the ridge at the base of the loop and enter the groove next anterior. In this manner particles while being carried dorsad are progressively moved toward the mouth. Nannoplankton forms (those less than about 25 m. in length) which may form as high as 80-90 of the food of the oyster,[4] are chiefly separated from dirt and carried to the mouth by this means.

Young oyster "spat" less than 3 mm. long, attached to glass slides were used for study of the part played by the filament of the gills and palps during feeding, and also for testing the effect of magnesium sulfate upon the reflex movements of the palps. The animals were studied whole under the microscope, the transparency of the shell permitting uninterrupted observation of the process. The gills and palps of the oyster at this stage consist of isolated filaments which are capable of independent movement.

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[2] Kellogg, J. L., *J. Morph.*, 1915, xxvi, 625.

[3] Allen, W. R., *Biol. Bull.*, 1914, xxvii, 127.

[4] Martin, W., *Bot. Gazette*, 1923, lxxv, 143.

In normal feeding the filaments of the palp are extended ventrad in the mantle chamber, their ends in close proximity to the outgoing ciliary tracts of the mantle. Particles reaching the palps via the dorsal groove were in the main accepted as were nearly all particles which succeeded in getting across the third filament from the posterior end.

In 1/20 sat. magnesium sulfate in water the filaments of the palps lost the power to erect, resulting in large masses of materials passing over the filaments to the mouth, eventually blocking it.

Feeding in the oyster is accomplished therefore through the delicate coordination of nervous, muscular, ciliary, and mucus secreting elements in which mechanical sorting of the materials plays the most important part.

The complete paper with the preceding one will appear in a zoological journal.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF COMMUNICATIONS.

One hundred and thirty-sixth meeting.

Cornell University Medical College, January 16, 1924.

President Jackson in the chair.

85 (2317)

Varieties of protein as factors in hippuric acid production.

By FRANK A. CSONKA (by invitation.)

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

Parker and Lusk were the first to point out the importance of the total N and hippuric acid ratio. Wiechowsky and Magnus-Levy observed a higher ratio than 4.7, which exists when the glycine in the body protein is utilized in the formation of hippuric acid, and they interpreted this increased ratio to a synthetic formation of glycine. There is no doubt that glycine is synthetized in the animal body. The new-born baby, for instance, lives on practically a glycine-free diet and yet increases its weight, and the newly developed tissue contains four per cent glycine which is not supplied by the food. On the other hand, if glycine synthesis plays an all-important rôle in hippuric acid formation when benzoic acid is administered, then we should not find any difference in the hippuric acid elimination when the protein supplied contains preformed glycine. The toxic effect noted after large quantities of benzoic acid are fed should occur always when a certain dosage is administered, that is, after the rate of glycine synthesis has reached its maximum and the amount produced is insufficient to counteract the toxic effect of benzoic acid. Our results do not confirm these theoretical arguments.

The hippuric acid elimination when casein, together with large quantities of benzoic acid, is given to a pig is practically identical with that when benzoate is given during starvation. Under like conditions the administration of meat protein results in a small increase in hippuric acid formation, while administration of gelatin, with its content of 25 per cent of glycine, causes a marked increase in the output of the substance.

When the pig was under the influence of benzoic acid certain reducing substances were eliminated in the urine, which were determined quantitatively, using Benedict's method for sugar. It was found that the quantity is largest when casein is the food given and smallest when gelatin is given, pointing to an inverse relation to the preformed glycine content of the protein ingested. The separation and identification of these glycuronates is being attempted. There is undoubtedly an increased N elimination when benzoic acid is administered, which supports the conclusion of those investigators who claim that an elevated protein metabolism follows its ingestion.

86 (2318)

Some cardio-vascular changes accompanying insulin hypoglycemia.

By D. J. EDWARDS, I. H. PAGE, and R. K. BROWN.

[*From the Department of Physiology, Cornell University Medical College, New York City.*]

It is evident from perfusion experiments on an isolated mammalian heart that this organ can functionate for several hours on its own stores of energy. Locke and Rosenheim[1] showed that the addition of glucose to the perfusion fluid produces a beneficial and sustaining effect upon the heart and that in turn this substance appeared to be actually utilized. More recently Hepburn and Latchford[2] have observed an increase in the sugar utilization of the isolated heart of over threefold when the pancreatic hormone was added to the perfusion fluid. These considerations indicate, therefore, that not only is the isolated heart capable of taking up carbohydrate for its activity but that its utilization of this substance is greatly augmented by insulin.

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[1] Locke and Rosenheim, *J. Physiol.*, 1904, xiv, 31.

[2] Hepburn and Latchford, *Am. J. Physiol*, 1922, lxii, 177.

The energy requirements of the heart beating outside of the body are obviously much less than they are when *in situ*. Can the heart continue functional activity without impairment very long under the conditions of high pressure and rate prevailing in the body, when its supply of carbohydrate is practically cut off from the circulating blood and its own capacity for using up this substance is increased?

In the present experiments hypoglycemia was produced by giving large doses (20-30 units per kg.) of insulin. The activity of the heart was studied by means of intraventricular pressure and ventricular volume curves, and the electrocardiogram. The vascular system was studied by means of optically recorded central arterial pulse tracings.

All of the experiments were performed on dogs. It was necessary to have an anesthetic that did not influence the blood sugar and for this we have obtained very satisfactory results with a new hypnotic iso-amyl-ethyl-barbituric acid.*

The mean blood pressure taken over periods of three to four hours shows a decline of not more than 20 to 30 mm. Hg. The intra-arterial pulse tracings, recorded from the carotid artery, exhibit only slight changes in form even in those instances where the blood sugar was maintained for two hours at a figure not exceeding 0.05 per cent. The change in pulse form consists of a more rapid primary rise followed immediately by the prediastolic fall. These characteristics of the pulse form become very marked in extreme relaxation of peripheral tone. Since these changes appear in a very moderate degree in these experiments we interpret it to mean that there is little primary change in the peripheral vessels accompanying the hypoglycemia.

The experimental results on the activity of the heart under low blood sugar seem to indicate a lessening of the dynamic function of this organ. The conduction function of the heart as shown by the electrocardiogram presents insignificant changes both in the auricular-ventricular conduction (P-R interval) and in the conduction through the ventricle (Q. R. S group). On the other hand there is shown in many instances a change in the form or an actual inversion of the T-wave during the progress of the experiment. The significance of this wave is still a matter of some doubt but there is much evidence in support of the view that it is an expression of the potential differences following muscular activity and as such undergoes modifications through influences that alter the muscular process.

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* Kindly supplied by Eli Lilly & Co.

In the records of intraventricular pressure we have an index of the dynamic capacity of the heart. The curves obtained in these experiments from hearts acting under a low blood sugar show a lengthening of the isometric period, or the phase of developing tension and a marked decrease in the maximum pressure developed during systole. We have also noted that the hearts in the animals with low blood sugar do not withstand the experimental procedures as well as those of normal animals. In some experiments where the heart was exhibiting a much weakened condition we have tried intravenous injections of glucose and have been able in this way to restore greatly its action as shown by the higher intraventricular pressure developed.

In two experiments we have recorded the volume changes of the heart by the cardiometer method as this technique seems less likely to interfere with the heart action. The results of these experiments confirm the changes already noted from the ventricular pressure records.

SUMMARY

In the hypoglycemia following the administration of large doses of insulin to dogs the experimental results show:

1. A small decline in mean blood pressure.

2. Slight alterations in the intra-arterial pulse curve.

3. No conduction time changes in the heart as signified by the electrocardiogram. The T-wave in some instances changed in form and in some instances inverted.

4. A lengthening of the period of developing tension of the ventricle and a decrease in the maximum pressure developed. Some restoration of ventricular activity by injecting glucose.

87 (2319)

Irregular series in protein precipitation.

By A. W. THOMAS and E. R. NORRIS.

[*From the Department of Chemistry, Columbia University, New York City.*]

The "irregular series" or "tolerance zone" in the precipitation of albumin with heavy metals has been observed by many investigators of the properties of proteins. Many theories have been advanced to account for the phenomenon but none of them have, up to the present time, given a satisfactory explanation.

The present work being conducted by the authors has linked the irregular series with the isoelectric point of the protein, thus accounting for the phenomenon by a known physical property of albumin rather than assuming the formation of a series of hypothetical compounds of varying composition and solubility.

In the experiments a solution of dried egg albumin was dialyzed against distilled water until practically ash free, filtered and kept under toluene. Series were then set up in fifteen cubic-centimeter graduated centrifuge tubes, such that the concentration of albumin remained constant in the final dilution, while the concentration of heavy metal salt varied. The tubes were allowed to stand thirty minutes, centrifuged twenty minutes and the volume of precipitate measured. The hydrogen ion concentration of the supernatant liquid was determined by means of the platinum electrode.

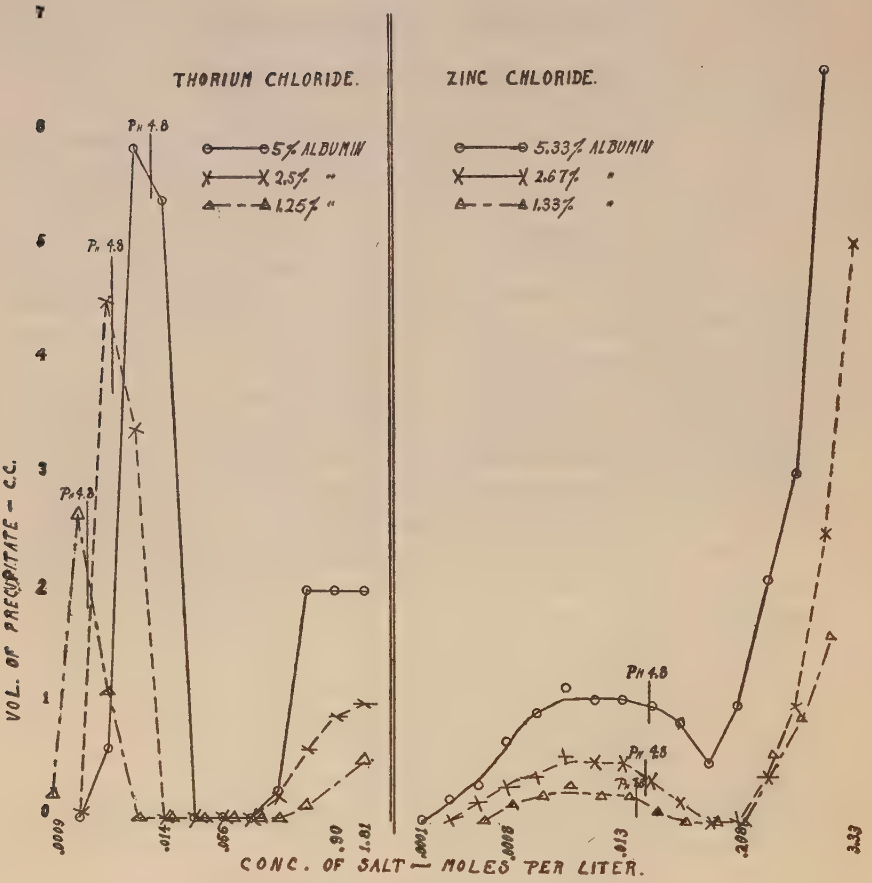
The curves shown in Figure 1 were obtained for thorium chloride and zinc chloride.

Cupric chloride and ferric chloride give curves similar to those of zinc chloride.

The aqueous solutions of heavy metal salts are decidedly acid due to the hydrolysis of the salt. The hydrogen ion concentration increases with increasing concentration of salt. In albumin solutions containing a low concentration of heavy metal salt there is an increasing precipitation with increasing salt concentration until the hydrogen ion concentration of the solution passes the isoelectric point of the protein (pH 4.8), when there is a marked falling off and disappearance of the precipitate.

Thus an insoluble heavy metal-albumin complex is formed

FIG. I



when the protein is on the alkaline side of its isoelectric point, and therefore acts as an anion, as the protein passes to the acid side of its isoelectric point its properties change to that of a cation and it is not precipitated by the heavy metal in dilute solutions. The precipitate formed in dilute solutions is readily soluble in dilute acids or in solutions of hydrogen ion concentration greater than $10^{-4.8}$.

The precipitate formed in concentrated solutions of the heavy metal salt, was not soluble either in water or dilute acids. When the precipitate was washed with dilute acid of approximately the hydrogen ion concentration in which it had been formed until the washings no longer gave a test for the heavy metal ion, a residue remained very similar in appearance to heat coagulated or denatured albumin and which after ignition did not give a positive test, for the heavy metal ion. The precipitation of albumin in concentrated salt solutions was not immediately complete but precipitate continued to form for a period of time which would indicate a secondary reaction or denaturing of the albumin. The precipitation with concentrated solutions of heavy metal salts is probably a salting out of the albumin followed by denaturing of the protein.

88 (2320)

Is the bacteriophage of d'Herelle volatile?

By J. BRONFENBRENNER and CHARLES KORB.

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

Following the description of the phenomenon of transmissable lysis of bacteria by d'Herelle in 1917, an enormous amount of evidence has been brought forward in support of his observations.

At the same time, however, there has also been an ever-growing tendency to dispute the parasitic nature of the active principle responsible for the lysis as postulated by d'Herelle.

In this connection, recent findings by Olsen and Yasaki[1] appeared to offer most important evidence which we attempted to corroborate.

Subjecting 18 hour cultures of so-called "bacteriophage" to distillation at 45-50° C. under reduced pressure, Olsen and Yasaki claim to have obtained a product capable of initiating transmissible lysis of cultures of susceptible bacteria. They conclude, therefore, that the "bacteriophage" is a volatile chemical substance and not a living parasite. Following their description of the experiment, we connected three flasks in series. The first, a distilling flask of one liter capacity, received 100 cc. of the filtrate from an 18 hour lysed culture of the colon bacillus (having a titer of 5×10^{-10} cc.) and was placed in a water bath kept at 45° C. With this was connected a similar distilling flask containing enough sterile distilled water to submerge the opening of the side-arm of the first distilling flask. This second flask was kept cool by means of an ice bath and was connected with a vacuum pump through a wash-bottle containing 25 cc. of sterile broth, also kept under ice.

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[1] Olsen and Yasaki, *Klin. Woch.*, 1923, xli, 1879.

Distillation was continued for from one to four hours in the three preliminary experiments with the result that in each case the second distilling flask yielded a distillate active against susceptible culture (lytic titer of from 1×10^{-4} cc. to 1×10^{-5} cc.).

The fact, however, that the titer of the original solution remained practically unchanged after distillation suggested that the activity of the distillate was due to droplet infection rather than to volatility of the active principle. This possibility was considered also by Olsen and Yasaki, but they finally decided that they were not dealing with droplet infection, since upon the addition of trypaflavin to the filtrate, they failed to obtain evidence of the dye in the active distillate. Instead of relying upon such indirect evidence, we decided to set up the apparatus in such a way as to exclude the possibility of the transfer of droplets. Accordingly, between the first (distilling) and the second (condensing) flasks a series of inverted funnel-shaped traps was interposed at a temperature of 45° C., so that any substance volatile at this temperature would be carried into the second flask, but any liquid particles would be filtered out of the current by the repeated impact against the glass surface.* In addition to titration of the distillate as well as of the residue, the traps were washed in measured amounts of sterile broth after each distillation, and washings thus obtained were titrated for their lytic activity. All the glassware and rubber used in these experiments was thoroughly sterilized by heat before use. Five distillations were carried out for varying lengths of time—two of them until complete dryness of the original filtrate. In all cases the distillates showed no activity whatever, the trap washings contained varying amounts of active lytic principle, and the residue retained its original activity, practically unchanged, even when distillation was carried to dryness. Our experiments fail to indicate volatility of the lytic principle of d'Herelle at 45-50° C.

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* It has occurred to us that a long tube filled with glass wool might be used in place of funnel traps.

89 (2321)

Effect of alcohol on the so-called bacteriophage of d'Herelle.

By J. BRONFENBRENNER and CHARLES KORB.

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

D'Herelle[1] has reported that by precipitating a culture of anti-dysentery bacteriophage with nine volumes of 96 per cent alcohol, decanting the supernatant fluid after 48 hours and re-dissolving the precipitate in saline, he obtained a substance slightly lytic for dysentery bacilli (titer 5 cc.). This lytic action, however, was not transmissible in series. D'Herelle concluded that the alcohol, while destroying the living ultra-microbe (bacteriophage), had precipitated its endo-enzyme.

Hauduroy,[2] repeating these experiments, obtained similar results by precipitating sterile bouillon with alcohol. He decided that the apparent lytic action of the precipitate obtained by d'Herelle was due to a bacteriostatic effect of the alcohol adsorbed by this precipitate. Arnold[3] was not able to obtain any active substance by precipitation with alcohol. More recently, Appelmans[4] failed to destroy completely the lytic activity of bacteriophage by exposure of bacteriophage to 50 per cent alcohol, but he does not state whether the activity of the precipitates was transmissible in series.

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[1] D'Herelle, F., The Bacteriophage, English Trans., Williams & Wilkins Co., 1922, 123.

[2] Hauduroy, P., Sur les Lysins du Bacteriophage d'Hérelle. *Compt. rend. Soc. Biol.*, 1922, ii, 964.

[3] Arnold, L., Basteriophage Phenomena. *J. Lab. and Clin. Med.*, 1923.

[4] Appelmans, R., Le Dosage du Bacteriophage. *Compt. rend. Soc. Biol.*, 1921, ii, 1098.

In the course of some experiments in which we had occasion to precipitate filtrates of so-called "bacteriophage" with alcohol, it was observed that in some instances the re-dissolved alcoholic precipitates manifested appreciable lytic action. Considering the divergent results reported by the previously mentioned observers and in view of the interest that the effect of alcohol on the activity of the lytic principle might have in connection with the question of the living nature of the bacteriophage, we have attempted to study this question further.

Several portions of active filtrate of 1 cc. each were placed in sterile centrifuge tubes. To each was added 10 cc. of 95 per cent alcohol and after thorough mixing of the contents, the tubes were kept for varying lengths of time both at room and at ice-box temperature. At stated intervals the contents of tubes were centrifuged and the liquid portion decanted off. The precipitates thus obtained were each dissolved in 1 cc. of sterile salt solution and the lytic titer determined. In order to ascertain whether the alcohol adsorbed by the precipitate exerted any bacteriostatic effect, the experiment was repeated after preliminary inactivation of the lytic filtrate at 90° C. for 20 minutes.

All dilutions for titration were made in 10 cc. of broth containing about 250,000,000 susceptible bacteria per cubic centimeter. Fresh pipettes were used for each dilution. The activity of dilutions exhibiting a doubtful degree of lysis was checked by transfer to agar slants. Those showing complete clearing were tested for serial transmission of lysis.

The experiments reported in this paper were made with two strains of dysentery bacteriophage,[5]—one active against *B. dysenteriae Shiga,* the other against *B. dysenteriae Flexner*—and with one strain of bacteriophage active against *B. coli.* Later, after the adaptation of the above lytic agents to other strains of the colon dysentery group, the experiments were repeated with the filtrates thus obtained. While in individual experiments there were observed some slight quantitative variations, the results were, in general, as follows:

Exposure of lytic filtrates to alcohol causes a rapid destruction of more than 99.9 per cent of the lytic substance within the first

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[5] These bacteriophages were isolated October, 1923, from the stools of patients at the Babies' Hospital. The specimens were obtained through the courtesy of Doctor Martha Wollstein.

15 minutes, both at room and ice-box temperature. Less than 0.1 per cent is recovered in the precipitate, while traces can be demonstrated in the supernatant alcohol. The titer of precipitates is usually about 1 x 10^{-5} cc. and that of the supernatant alcohol about 1 x 10^{-2} cc. At room temperature the deterioration of the lytic principle proceeds very rapidly with the further exposure to alcohol so that at the end of from three to eight hours not a trace of the lytic action can be detected, either in the precipitates or in the supernatant alcohol. At ice-box temperature, on the other hand, the lytic titer of 1 x 10^{-5} cc. remains unchanged for at least five to six days and begins to deteriorate slowly with further exposure to the action of alcohol. This lytic action is not due to the bacteriostatic effect of alcohol but is transmissible in series exactly as in the case of the original filtrates.

90 (2322)

Kidney changes in pyloric obstruction.

By F. D. ZEMAN, WM. FRIEDMAN and L. T. MANN (by invitation.)

[*From the Pathological Laboratory, Mount Sinai Hospital, New York City.*]

Four cases of pyloric obstruction have been studied with particular reference to the microscopic changes in the kidney. Of the four, two showed typical tetany, presumably of gastric origin. One of these was under observation only 24 hours, and was unoperated, dying shortly after admission to the hospital. The other case, in which a huge gastrectasia was demonstrated by Roentgen-ray, developed tetany following operative resection of stomach. The two cases without tetany had symtoms of gastric ulcer with signs of obstruction, and were treated surgically.

The kidney changes were not appreciated before death, and were only discovered in course of routine microscopical examination of autopsy material. The four cases show almost identical kidney changes. These consist in degeneration of cells lining the spiral and terminal straight portions of the first convoluted tubules. In many sections these cells are necrotic and infiltrated by granules staining dark blue with hematoxylin, which merge into larger masses, entirely blocking lumina of tubules. These blue staining granules we have interpreted as calcium salts whose staining reaction with hematoxylin is generally regarded as highly characteristic. Treatment of sections with 5 per cent nitric acid for 20 to 30 minutes and then staining with hematoxylin, showed complete disappearance of blue-staining material.

The changes we have found in these kidneys have long been regarded as a frequent concomitant or result of poisoning with mercuric chloride. The similarity is so marked that in our first case we were for a time entirely misled.

It must be emphasized that we do not consider these kidney changes as evidences of a nephritis. The cardinal signs and criteria of either an acute or chronic inflammatory lesion are entirely lacking. The changes we have observed are entirely of a degenerative nature, and as such are best designated by the term toxic degenerative nephrosis.

The observation that gastric tetany may be accompanied by renal changes was first made by Nazzari, who in Policlinico, 1904, reported two cases of gastric tetany with calcification of renal convoluted tubules. He had observed a similar picture in cases of mushroom poisoning and held that his findings pointed to the toxic origin of tetany.

This work remained without confirmation until very recently when a group of workers from the Mayo Clinic, Brown, Hartman, Eusterman and Rowntree, reported eleven cases of duodenal toxemia resulting from high intestinal obstruction in which they found renal changes, which they described as either a diffuse nephritis or a nephrosis. Calcification occurred in several of their cases, but they attribute this to administration of calcium by mouth. While their work is complete, with full chemical and clinical observations, we cannot as yet subscribe to their interpretation of the findings which are in general similar to our own.

Notwithstanding the fact that three of our four cases received calcium internally we do not feel that this has any direct bearing on the character of the kidney lesions described.

We feel that the primary factor in our observations is pyloric or high duodenal obstruction in association with repeated vomiting, to which the tetany and kidney changes appear to be secondary. The mechanism leading to the kidney damage we are unable to explain satisfactorily at present. Sections of stomach and other organs in our series showed no deposit of calcium such as occurs in metastatic calcification where the amount of circulating calcium is increased.

These observations have served as a basis for further investigation, and are being tested in the experimental studies which we are now undertaking. By tying off the pylorus of cats, it has been possible to reproduce experimentally kidney lesions similar to those described.

91 (2323)

The skopometer: An instrument for measuring color, cloudiness, and other optical phenomena of liquids.

By WILLIAM G. EXTON (by invitation).

[*From the Laboratory of The Prudential Insurance Company, Newark, N. J.*]

The Skopometer was designed for measuring color, cloudiness, and other optical phenomena of liquids without the necessity of using standards for comparison.

Color is measured in terms of absorption by determining the extinction coefficient of the liquid with the aid of a monochromatic light filter complimentary to its color.

The construction permits measurements of cloudiness by means of several different optical principles. Denser clouds are conveniently measured by determining the height of the column of liquid necessary to extinguish a light image of standardized intensity by means of a target such as a platinized glass disc with a transparent line or design of given dimensions. The target may be used in various ways and illuminated by either incident, grazing or transmitted light.

Lighter clouds are measured photometrically by matching the light incident for the target with the light reflected or scattered from the particles in suspension. For very small quantities of liquid the standard target is modified by the cloudiness of a 10 mm. column of the liquid and matched photometrically with cross illumination through the plunger, the visual effect in all cases being that of disappearance of the target.

By means of a standard solution and a complimentary light filter, illumination is standardized by bringing the light of the special lamp to a distance corresponding with the extinction coefficient.

92 (2324)

The influence of the ingestion of potassium chlorate, sodium chloride and water on the oxygen capacity of the blood.

By VICTOR ROSS (by invitation).

[*From the Research Laboratories of Lehn and Fink, Inc., Bloomfield, N. J.*]

The experiments reported here were undertaken in order to compare the effects of the ingestion of potassium chlorate, sodium chloride and water on the oxygen binding capacity (hemoglobin) of the blood. Dogs were used. The last meal was given 18 to 24 hours before the experiment was begun but the animals had free access to water at all times except during the course of the experiment when they were kept in metabolism cages. In this manner it was hoped that when the experiments were begun the hemoglobin of the dogs' blood would be at the physiological level and that normal variations would be avoided as far as possible.

A dose of 0.5 gm. of potassium chlorate per kilo of body weight was given by stomach tube in one per cent solution (equivalent to 50 cc. of water per kilo). At the end of two, four and six hours blood was taken from the jugular vein, following which the dog was given 25 cc. of water per kilo. This made a total of 125 cc. per kilo. A final blood sample was taken 24 hours after the beginning of the experiment. Blood oxygen capacity determinations were made by the Van Slyke method.

The results show that there may be no fall or a slight fall in oxygen capacity *after two hours* in those cases where the oxygen capacity before feeding potassium chlorate was less than 20 volume per cent. In these cases, however, there was a fall varying from 0.17 to 1.6 volume per cent at the end of *four to six hours.* By this time the dogs had had, in addition to the potassium chlorate solution, one or two doses of water (25 cc. per kilo), as explained above. On the other hand, animals showing an initially higher oxygen capacity (21.5 to 23 volume per cent) suffer a fall of from 1.6 to 2 volume per cent after *two hours.* In general the higher the oxygen capacity before feeding the greater and more rapid the fall. Since no methemoglobin could be detected in the spectroscope the drop in the oxygen capacity is not the result of the conversion of hemoglobin to methemoglobin.

When sodium chloride was given, 0.5 gm. per kilo of body weight in one per cent solution, followed at two hour intervals by 25 cc. of water per kilo, similar effects on the blood oxygen capacity were observed. Here also, the greater the original value the larger the drop and the more sudden. These results for sodium chloride are similar to those obtained by Haldane and Priestley[1, 2] in their hemoglobin measurements when they fed solutions of sodium chloride to humans.

When water alone is given, 50 cc. per kilo of body weight, followed at two-hour intervals by 25 cc. per kilo, the blood oxygen capacity falls in the same manner as when solutions of potassium chlorate or sodium chloride are fed. Although only two experiments were performed with water the results are clear. A drop of 1.5 volume per cent and of 2 colume per cent was observed, the dog with the originally higher value suffering the greater fall. Haldane and Priestley found that water ingestion did not alter the hemoglobin concentration of human beings. The discrepancy may be due to the fact that our dogs received proportionately much larger quantities of water. A 70 kilo man would, on our basis, take 3,500 cc. of water *within a very few minutes.*

The results indicate that (*a*) the degree of temporary dilution of the blood as evidenced by the lowered oxygen capacity values is approximately the same for solutions of potassium chlorate

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[1] Haldane, J. S., and Priestley, J. G., *J. Physiol.*, 1915-1916, l, 296.

[2] Priestley, J. G., *J. Physiol.*, 1915-1916, l, 304.

and sodium chloride as for water alone, (*b*) since the fall in oxygen capacity is not so great for dogs with lower original values as for dogs with greater values, there is an arrangement for protecting the concentration of the blood against disturbing influences, (*c*) no methemoglobin is formed when potassium chlorate is fed under the above named conditions.

93 (2325)

Studies concerning the significance of Streptococcus hemolyticus in scarlet fever.

By A. R. DOCHEZ
With assistance of LILLIAN SHERMAN.

[*From the Department of Practice of Medicine, Presbyterian Hospital, Columbia University, New York City.*]

The more or less constant presence of *Streptococcus hemolyticus* in the throats of individuals suffering from scarlet fever has been generally recognized for many years. This organism is also the preponderant causative agent of such septic complications of the disease as otitis media, adenitis, interstital nephritis, arthritis, and septicemia. Such widespread and general relationship of streptococcus to scarlet fever naturally gave rise to the view, supported by a number of investigators, that streptococcus might be the etiological agent of the disease. Belief in the validity of this conception led to the preparation and trial of antistreptococcic sera for therapeutic purposes. For some of these efficacious results have been claimed. On the other hand another group of investigators has asserted that streptococcus bears only a secondary relationship to scarlet fever and cannot be assigned the principal causative role. Discussion of this question was carried on for many years and became part of the larger controversy concerning the nature of the whole group of organisms generically designated as *S. hemolyticus*. The balance was finally tipped against the etiological importance of *S. hemolyticus* by Jochmann, who claimed that it is unreasonable to suppose that a specific disease such as scarlet fever can be caused by an organism giving rise to such varied manifestations as *S. hemolyticus* and furthermore that the presence of this organism cannot be demonstrated in certain rapidly fatal malignant instances of the diseases.

In 1919 Dochez, Avery and Lansfield developed a technique for the differentiation of biological types of *S. hemolyticus.* This discovery led to a reexamination of the type of streptococcus associated with scarlet fever. As a result of these studies it has been found by Bliss, by Tunnicliff, by Gordon, and by Stevens and Dochez that the type of hemolytic streptococcus found in the throats of scarlet fever patients is in general a specific type readily distinguishable from the types of *S. hemolyticus.* causing other kinds of angina and septic conditions in general. Furthermore this specific streptococcus has been isolated from the local wound in wound scarlet, from the infected burn in burn scarlet, from the lochial discharge in puerperal scarlet and from both patients and contaminated milk in a milk-borne epidemic of scarlet fever. More careful study of the throat secretions of patients with scarlet fever has demonstrated the organism to be present at some time during the disease in 100 per cent of instances. It would seem therefore that the two main objections of Jochmann to the etiological significance of *S. hemolyticus* in scarlet fever have been answered. As a result of these studies the question again assumes renewed importance.

Ever since the initiation of these studies we have made more or less continued efforts to produce in animals, with a scarlatinal type of *S. hemolyticus,* a disease resembling scarlet fever. At first these efforts met with questionable success. More recently, with a special technique we have been more successful and have produced in guinea pigs a condition resembling in its main features certain of the phenomena of scarlet fever. The animals develop fever, leucocytosis, lose weight; on the second and third day show a transient erythematous flush; and on the 8th to the 12th day more or less general desquamation appears, most conspicuously on the pads of the feet. Dick and Dick have recently published the results of some human inoculations with *S. hemolyticus* which were followed by what may have been mild attacks of scarlet fever.

In view of the similarity to scarlet fever of the symptom complex produced in guinea pigs we decided to immunize a horse by a similar procedure. We have recently obtained from this horse an immune serum which has been tested by Dr. Francis G. Blake for its capacity to neutralize the rash locally in the skin in human cases of scarlet fever. In all the cases tested so far a positive neutralization has occurred which is somewhat more conspicuous than that obtained under similar circumstances from the use of convalescent scarlatinal serum. We propose in the near future to test the serum therapeutically. Such a sequence of observations undoubtedly again throws the balance of evidence in favor of *S. hemolyticus* as the causative agent of scarlet fever. It would seem that the disease may not be unlike diphtheria in that the principal localization of the infection is in the throat where the organism produces a toxin which gives rise to the general symptoms and the rash. The immunity produced is in all likelihood antitoxic in character and the blanching reaction represents the neutralization of the toxin *in situ.*

94 (2326)

On the pharmacological action of the anti-rachitic active principle of cod liver oil.

By T. F. ZUCKER and M. J. MATZNER.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

We pointed out some time ago that the diets used to produce rickets in animals have an appreciable excess of base over acid and that rickets is not produced if such diets are made more acid. We also noted that the hydrogen ion concentration of infants' stomach contents is lower than that of adults, and that a further depression may appreciably decrease the amount of soluble calcium and phosphoric acid available for absorption. In looking for a possibility of explaining the action of cod liver oil in rickets, we studied its effect on the reaction of the intestinal contents. The feces of rats on the rickets producing diet No. 84, used in this laboratory, when made into a suspension with water, give a pH of 7.4 to 8.0 with an average of about 7.6. When such rats are given the active principle of cod liver oil which we have described or cod liver oil itself, the reaction of the feces changes in a few days to the acid side, usually to about pH 6.2 or even as far as pH 5.7. Controls with cotton seed oil give an unchanged alkaline reaction of the feces.

When a solution of the active principle in olive oil or in glycerol is injected into rachitic rats subcutaneously, it does not produce healing or change the pH value of the feces, provided measures are taken to prevent the licking of the site of injection. (We apply a drop of collodion with a little picric acid dissolved in it.) We thus have additional evidence that the action of the active principle is in the gastro-intestinal tract. Orr et al.[1] on the basis of metabolism experiments on infants have also taken the viewpoint that the primary disturbance in rickets is in the gastro-intestinal tract.

Now it remained to see whether the rickets curing effect of light may be reduced to the same basis. Rats on diet 84 exposed to a moderate amount of sunlight showing by x-ray an incomplete prevention of rickets give a feces reaction of about pH 6.7, while another group liberally treated with the mercury-vapor lamp showed complete prevention of rickets and a feces reaction of about pH 6.4. Babbot and others[2] have shown that in infantile tetany the pH of the stomach contents is high and that treatment, for instance with the mercury-vapor lamp, tends to restore it to the more acid normal value.

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[1] Orr, Holt, Wilkins, and Boone, *Am. J. Dis. Child.*, 1923, xxvi, 302.

[2] Babbott, Johnston, and Haskins, *Am. J. Dis. Child.*, 1923, xxvi, 486.

95 (2327)

A medium for the inhibition of spreaders and the differentiation of B. coli and B. aerogenes.

By CHARLES EDWARD SKINNER and T. J. MURRAY.

[*From the Department of Bacteriology, Rutgers College, New Brunswick, N. J.*]

In a study of a project under way a culture medium was developed that differentiated between *Bacillus coli* and *Bacillus aerogenes* and inhibited spore formers (spreaders). This medium is Levine's eosin methylene blue agar to which is added 1/100,000 of crystal violet.

In testing this triple dye agar out, 50 spreaders isolated from soil and 100 strains of the "Colon Bacilli", isolated from a variety of sources, were streaked on this medium. None of the "spreaders" developed in 24 hours; in 48 hours, 21 developed slightly; the remainder never developed. No deleterious effects were shown the "Colon Bacilli" of which 73 were of *B. coli* and 27 were *B. aerogenes,* separated on the basis of the Voges-Proskauer reaction.

The *B. coli* and *B. aerogenes* colonies are very typical in 48 hours. *B. coli* colonies are black or dark bluish violet by transmitted or reflected light, with dark centers reaching to the edge or nearly so. They tend to show a decided green metallic sheen. They show no tendency to run together.

B. aerogenes colonies are pink or light lavender by reflected light, by transmitted light, dark centers not reaching more than half way to the edge. The colonies are sticky, showing a decided tendency to run together. There is no metallic sheen.

Samples of soil, feces, sewage and a polluted stream were inoculated in lactose bouillon for enrichment. Streaks were made on the triple dye agar and colonies studied and classified as *B. coli* or *B. aerogenes.* These colonies were also inoculated into lactose bouillon for confirmation and into dextrose broth for the Voges-Proskauer test. Two hundred and thirty-five colonies were called *B. coli* and 99 *B. aerogenes.* By the above tests 222 of the 235 colonies of 94.4 per cent were actually *B. coli,* while 92 of the 99 or 93 per cent colonies were actually *B. aerogenes.*

96 (2328)

Vitamin studies. X. Feeding technique in vitamin studies.*

By R. ADAMS DUTCHER and EMMA FRANCIS.

[*From the Department of Agricultural Chemistry, Pennsylvania State College, State College, Pa.*]

The work described in this paper is presented with the view of re-emphasizing the importance of the use of false screen bottomed cages in vitamin studies when rats are used as the experimental animals. Steenbock, Sell and Nelson[1] have pointed out that rats which do not have access to their own feces require a larger proportion of vitamin B in the basal ration than those rats which ingest variable amounts of excretory material daily. In our studies of vitamin B in the past (using diets deficient in this vitamin), we have often been unable to account for the lack of uniformity in the development of animals from the same litters on identical diets. In fact certain animals grew satisfactorily for many weeks on a vitamin B deficient ration. In watching the animals over relatively long periods it was observed that those animals whose food intakes and growth curves were most satisfactory were in the habit of ingesting relatively large amounts of excretory material; for example we have noted a few animals which would consume as many as 15 to 20 pieces of feces in the space of eight or ten hours.

In order to study the matter more thoroughly 63 rats were placed on experiment in individual cages. All animals received a basal ration consisting of casein 18, salts 3, agar 2, dextrin 77 and 5 drops of cod liver oil daily. Iodine was furnished in the drinking water. A number of the animals were placed in cages without screens while the remainder were distributed in cages containing false bottoms made of galvanized wire screens (3 meshes to the inch). The false bottoms were constructed about 1 1/4 inches above the cage pans in order that the particles of feces would fall through the screens to the pans below where the rats could not reach them. As indicated in the chart, weekly records were taken of the amount of food eaten, but it was impossible to keep an accurate record of the weight of fecal material ingested. In order, however, that we might have a fairly definite idea of the feces intake, certain rats were placed on screens, and feces from definite sources were fed. In some cases the feces were collected from rats which were on diets rich in vitamin B, while in other cases feces were collected from rats on diets containing little or none of this accessory food substance.

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[1] Steenbock, H., Sell, M. T., and Jones, J. H., *J. Biol. Chem.*, 1923, lv, 399.

* Published as Paper No. 6, Department of Agricultural Chemistry, Pennsylvania State College.

Some of the animals were placed on screens from the start and later the screens were removed in order to provide ready access to the fecal particles. Other rats were allowed access to feces from the start and later the screens were introduced.

In order to conserve space we have chosen a few of the most striking curves, placing them in a single chart for comparison. Certain curves were chosen, not because they are necessarily representative, but rather to show to what extremes animal experiments may go when seemingly unimportant details in technique are neglected.

The curve given for rat 392 is representative of all animals which were on screens without access to feces from the beginning of the experiment. It is evident that there is little, if any, vitamin B in the basal ration. When vitamin-rich feces from another rat were fed to rat 392 (see arrow) the response in food intake and growth was immediate. Although rats 292, 293, 296 and 299 received the same ration as rat 392, it is interesting to note that they were able to grow at a fairly satisfactory rate for from five to seven months, in spite of the fact that the ration was deficient in vitamin B. When screens were introduced in the cages containing rats 292 and 293, the fall in the food intake and body weight was very marked. On the 230th day vitamin-rich feces, obtained from rats on a vitamin-rich diet, were placed in dishes and offered to rats 292 and 293. The increase in food intake and body weight is ample proof that the feces possessed a stimulatory effect.

The curves of rats 299 and 301 tell the same story up to the point where the screens were introduced. When vitamin-rich feces were fed, the responses were very marked while the reverse was true when vitamin-poor feces were fed (see curve for

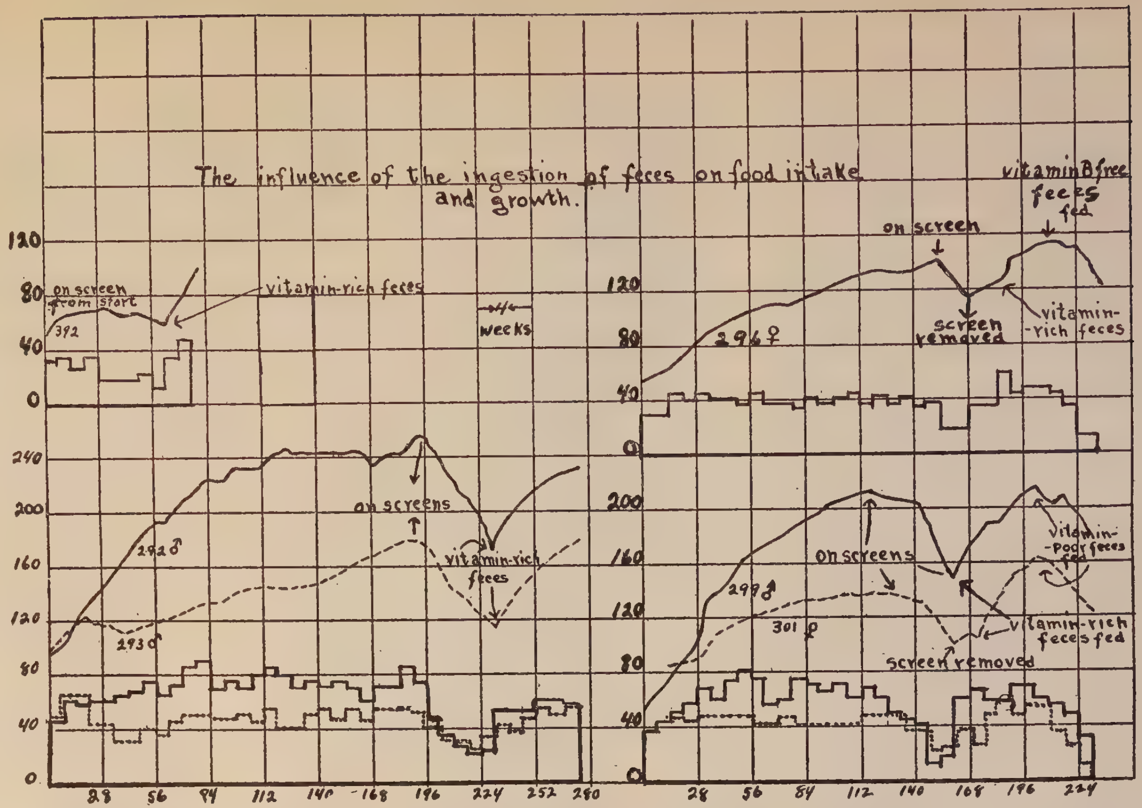

Chart showing the deportment of albino rats on diets deficient in vitamin B, with and without the use of false screen bottoms in the cages. Those rats which consumed large quantities of excreta were able to grow well—due, apparently, to the tissue vitamin stored in the body previous to the feeding trials. It would appear that this food hormone can be used over and over again. When rats are placed on screens appetite and body weight fall almost immediately. Rats cannot store vitamin B for long periods. This is indicated by the fact that the feces of the rats (on screens) have lost this vitamin within two weeks time.

rats 296, 299 and 301). The parallelism between food intake and increase in body weight is interesting and emphasizes the necessity of possessing data on food intake of individual animals. This, in our opinion, has considerable advantage over the "group feeding" method inasmuch as it often aids in the interpretation of data. For example we have found it advisable to exclude all animals in any experimental group that have shown sub-normal appetites when compared with their litter mates receiving the same ration. This would be impossible if the feeding was conducted with several animals in a cage.

Curves 292, 293, 299 and 301 emphasize the point just mentioned. Rats may be reared in the same environment and on the same rations and yet deport themselves differently, depending upon the amount of food that the animal normally desires to ingest. In other words, they will grow in proportion to the natural appetite that they possess.

We have also been able to corroborate the finding of Steenbock with reference to the storage of vitamin B. It is quite evident that the rat is able to store sufficient vitamin in the tissues to carry the animal over several months, provided a large proportion of the feces are eaten. In other words it would seem that the stimulatory substance can be stored, utilized, voided, re-ingested, and the cycle continued for a considerable time, indicating that the demand for vitamin B is urgent but that the quantity needed is not large. On the other hand, if rats are placed on screens for two weeks and then the screen removed, allowing the rat to commence feces eating at that point, the feces seem to have lost their stimulatory properties. This would indicate that the rat does not possess the power to store the vitamin for long periods.

CONCLUSIONS.

Rats are prone, if they have the opportunity, to consume varying quantities of excreta, the ingestion of which seems to keep up a sufficient supply of some stimulatory material (probably vitamin B) to vitiate certain types of experimental work. Just how serious the work of the past has been affected it is difficult to tell. Certainly it will be necessary to repeat certain types of work.

The importance of food intake records for individual animals has been emphasized.

Evidence is cited to show that the rat is limited in its ability to store vitamin B.

97 (2329)

The influence of the liver on the chemical regulation of the heart.

By LEON ASHER.

[*From the Physiological Institution, Berne, Switzerland.*]

The liver was the first tissue to which the function of internal secretion was ascribed by the genius of Claude Bernard. The liver is further the place of the most manifold and intense chemical processes in the animal body and no single cellular unit is in possession of so many enzymes as the liver cell. Yet as to internal secretion of the liver our knowledge is very scanty and modern text-books of endocrinology make little mention of the liver in this connection.

Dr. Takahashi and myself have made a series of experiments on the question as to whether the liver contributes towards the chemical regulation of the heart-beat. We have employed methods which we have been using for related questions since the year 1917 in studying the possible chemical basis of the action of antagonistic nerves, such as those regulating the beat of the heart. We used frog's heart as this is very sensitive to slight changes in chemical conditions and allows easy investigation under controlled conditions without any other influences hampering the results. Either a normal Ringer's solution, or Ringer's solution mixed with blood, was perfused from a small collecting vessel by way of the vena cava interior through the heart and returned from the aorta to the collecting vessel. We used this method in order to be able to work with small quantities of the fluid and to collect any substances which may be formed during the course of the experiment. The beat of the heart is recorded by aid of a suspension lever. Both vagi are laid on electrodes and stimulated from a graduated Kronecker induction coil to ascertain the excitability of the vagi. A second frog is taken and the heart perfused in the same way with the only difference that the fluid coming from the collecting vessel is made to pass the liver first, from whence it enters the heart. The fluid which has repeatedly passed liver and heart during a period of half an hour is then transferred to the other heart. The effects of this fluid which has passed the liver on the heart are very marked. The heart beats become augmented in size and in frequency and in harmony with these facts the excitability of the vagi is lowered. In certain especially successful experiments, where the conditions were favored by the possibility of stimulating with very weak stimuli, during the passage of the fluid having previously passed the liver, a stimulation of the vagi which before had caused inhibition now evokes acceleration of the heart. The action was very much like that of a slight dose of atropin which by paralyzing the inhibitory fibres of the frog's vagi, allows the sympathetic augmentary fibres to prevail. In certain, not very frequent cases, there may occur a reversed effect, *i. e.,* an increase of vagus excitability probably due to functional adaptation, the importance of which for the understanding of hormonal activity I tried to insist upon in my Harvey Lecture, delivered February 24, 1923. But the main effect is a preponderance of the sympathetic side of the heart's activity.

Various conditions must be fulfilled to bring out the effects here preliminarily reported. Using the frog's heart, it is better to employ a mixture of blood and Ringer's solution. The perfused fluid should be continuously aerated. To exclude a difference of the two fluids compared, they are brought to the same content of sugar.

A series of experiments was also performed on the tortoise heart. Two animals were used and the methods were essentially the same as in the former experiments. Conditions of the vagi are somewhat different from those in the frog. In perfusing the liver it is better to abstain from using defibrinated blood, as the blood which has passed the tortoise liver will always coagulate, evidently by the rapid formation of fibrinogen in the tortoise liver. The results in these experiments are the same as in the former series. The fluid which has passed the liver contains something which has an augmentor action on the heart and decreases the inhibitory action of the vagi.

In the experiments of both series the primary conditions were restored by perfusing a fluid which had not before passed the liver. These experiments, of which a more detailed report will appear in another place, seem to show that the liver contributes towards the chemical regulation of the heart-beat, acting on the neuro-muscular mechanisms of the heart. One may also call it a hormonal control of the heart by the liver, and if one accepts this mode of expressing the facts, the experiments recorded demonstrate an internal secretion of the liver. For the moment I prefer to speak of chemical regulation, the nature of which we are trying to study by further experiments.

I should not like to close this preliminary communication without reminding of the interesting and highly suggestive experiments of Cannon, who found in the mammal that acceleration of the heart, evoked by reflex stimulation, could persist in a certain degree even after extirpation of the adrenal glands —as was also found by Stewart—and was due according to his experiments to the influence of the liver.

98 (2330)

A note on the estimation of carbon dioxide in serum by the Van Slyke method in the presence of ether.

By J. HAROLD AUSTIN.

[*From the Department of Research Medicine, University of Pennsylvania, Philadelphia, Pa.*]

When serum contains an amount of ether such as is present in an animal fully anesthetized, an appreciable error is introduced into the analysis of the CO_2 content by the Van Slyke method, using the ordinary technique with either the constant pressure or the constant volume machine. This is due to the extraction of a considerable part of the ether during the initial extraction of the gases and to the reabsorption of most of this ether by physical solution during the absorption of the CO_2 with alkali. By the ordinary technique the apparent CO_2 content may be too high by as much as 10 volumes per cent.

A modification of the technique has been developed which makes it possible to measure the CO_2 in the presence of ether. This consists essentially of reextraction of the alkaline solution after absorption of the CO_2 with alkali. The CO_2 remains held by the alkali but the ether is again extracted in part into the gas phase. An empirical correction must be introduced to correct for a greater solubility of the ether in the acidified solution than in the alkaline solution.

Abstracts of Communications.

Pacific Coast Branch.

Thirty-eighth meeting.

Stanford University, California, October 27, 1923.

99 (2331)

Estimations of the transport numbers in solutions of sodium and potassium caseinate.

By D. M. GREENBERG and CARL L. A. SCHMIDT.

[*From the Department of Biochemistry and Pharmacology of the University of California, Berkeley, Calif.*]

Experimental work has been carried out to determine according to the well known method of Hittorf the transport numbers of caesium, sodium and potassium caseinates. Constancy of pH was maintained throughout the experiment by the aid of a nonpolarizable cathode. Casein was deposited on a platinum wire which constituted the anode. The experimental results indicate that in the solutions which were employed the current is carried by the caesium, sodium or the potassium as cations and the casein as anion. These results are not in harmony with the hypothesis which postulates that the protein ionizes to give two complex protein ions rather than an inorganic ion and a protein ion. The experimental data will be published in detail in the near future.

100 (2332)

Recovery of Bacillus histolyticus from human feces.

By IVAN C. HALL.

[*From the University of California, Berkeley, Calif.*]

Several months ago, Miss Peterson and I reported finding *B. histolyticus* in a sample of arable soil from California.[1] This was one of the few times that this organism has been recovered from a source other than war wounds, and, we believe, the first authentic report of its occurrence outside of the war zone, and particularly in America.

I have just recently isolated another strain of *B. histolyticus* (No. 290) from a human stool, which suggests that this highly pathogenic organism, like *B. Welchi, B. tetani,* and *B. botulinus,* at least occasionally, if not usually, makes its home in the intestinal tract of man and possibly other animals and gains access to the soil in part from fecal sources. There seems to be no previous record of the recovery of *B. histolyticus* directly from the intestinal tract or from feces.

The stool from which it came was obtained March 24, 1923, from a healthy young man, a prisoner in San Quentin prison, serving with several others in a diet squad under the direction of Dr. G. W. Clark in his study of calcium metabolism in relation to dental diseases. This squad had just completed an eight weeks' period in which the weekly menu was typified by that of this man in the last week as follows, in grams: bread 1500, granulated sugar 415, sugar candy 210, shredded wheat 335, graham crackers 700, meat 758, milk 3500, raisins 420, tomatoes 1021, salt 15, butter 415.

The stool was collected aseptically in a large sterilized evaporating dish and was kept covered in an ice chest until sampling time, which was about two hours afterwards. The stool was moderate in size, soft, but well formed. It contained in addition to *B. histolyticus,* also *Bact. coli, B. mesentericus* and *B. Welchi. Bact. coli* was recovered aerobically from an unheated suspension; the spore formers were recovered from a suspension heated to 80° C. for 20 minutes, using deep brain medium for the initial culture and deep agar for isolation of the anaerobes.

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[1] Peterson, E., and Hall, I. C., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 502.

This culture of *B. histolyticus* shows all of the usual morphologic, cultural and pathogenic characteristics of the species.[2] It also grows definitely though delicately upon the surface of meat infusion blood agar slants exposed to the air. Such aerobic cultures failed to show hemolysis as do anaerobic cultures, and the stained organisms from them were atypical fusiform rods with few spores as in our former observations. This species, though not always strictly anaerobic in the sense of refusing to grow upon the surface of solid media, evidently prefers anaerobic conditions, as shown by these facts, as well as in the greater size and number of colonies in the depths of deep agar shake cultures.

This strain is exceedingly virulent; the leg of a guinea pig injected intramuscularly with 2 cc. of a 24 hour glucose broth culture was on the following day almost completely disarticulated and denuded of flesh from the knee down in addition to an area of about 15 sq. cm. on the abdomen from which the skin had sloughed.

The finding of this species in human feces, as well as in cultivated soil, establishes evidence, albeit a slender thread, to explain its occurrence in wounds where conditions are favorable for contamination with fecally polluted soil.

101 (2333)

Studies on inorganic salt metabolism.

By MARTHA R. JONES (by invitation).
With the co-operation of LAURA JAMES and CURTIS E. SMITH.*

[*From the Department of Pediatrics*† *and the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco, Calif.*]

Studies on inorganic salt metabolism in dogs and rats which have been in progress in this laboratory during the past two years indicate that a bone condition comparable in all essential respects to that seen in rachitic children may be produced in young puppies on a diet, which, as far as we have been able to determine, is adequate in respect to protein, fat, carbohydrate, inorganic salts and vitamins but possesses a high potential alkalinity; while parallel experiments with the same diets on rats have invariably resulted in normal bone development.

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[2] Hall, I. C., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 501.

* From the Department of Household Science, University of California, Berkeley, and the Department of Pathology, University of California Medical School, San Francisco.

† Part of the expense of this investigation is being borne by a grant from the W. H. Crocker Fund for Research in Pediatrics.

Our basal diet consisted of bread, cooked lean beef, skimmed milk powder, butter fat and fresh orange juice made into a mush with distilled water. To this was added a salt mixture, the composition of which was based on ash analyses of cow's milk, except that the phosphorus was omitted and different proportions of calcium were used. The phosphorus content of the diets was varied by adding dibasic potassium phosphate to the above salt mixture, and the degree of alkalinity regulated by the addition of HCl. The first lot of salt mixture prepared proved to be alkaline in reaction and when added to the food mixture as described above and fed to six young puppies resulted in severe rickets in all cases, our diagnosis being based upon blood and bone analyses for calcium and phosphorus, radiographic, clinical and histological evidence. The parallel experiment on rats was negative. Another group of three puppies of the same litter received the identical diet with the addition of 0.8 gm. of phosphorus daily. They too developed rickets, the clinical signs appearing earlier than in the former group, while the rats fed similarly were normal. The experiment was repeated as nearly as possible a year later on two litters of puppies, 11 in number, except that the excess of alkali was neutralized with HCl. No signs of rickets were detected at any time in either puppies or rats during the experimental period of six months. We again produced well marked rickets in three puppies of the same litter on the basal diet with the addition of an alkaline salt mixture, the calcium content of which was higher than that of the first two lots, and cured two of these simply by adding sufficient HCl to the salt mixture to neutralize the excess of alkali, while the third animal was kept on the original diet and became steadily more rachitic. Here again the rats failed to develop any signs of the disease.

The fact that different litters of puppies and even individuals of the same litter vary tremendously in their susceptibility to rickets is generally conceded. A factor which has not been stressed in the reports of recent researches on the etiology of rickets but which we have found to have a very important bearing on the ease and rapidity with which the disease may be induced in an animal, is the diet of the mother during pregnancy. In our experience, brood bitches which are fed on diets deficient in calcium give birth to litters which have marked rachitic tendency—some individuals developing clinical signs even before weaning. In these puppies there is a marked retardation in the appearance of the epiphyses and carpal bones of the feet and remarkable skeletal changes can be demonstrated by radiographs after two weeks on the experimental diet. Another brood bitch living under comparable conditions and fed in a manner similar to those mentioned above except that a well balanced inorganic salt mixture was added to the diet gave birth to normal young, our conclusions being based on the number and size of the epiphyses and carpal bones appearing in the fore feet at different ages.

It has been shown by a number of investigators that in active rickets the distribution of calcium and phosphorus between urine and feces is different from that which occurs in normal individuals. The amount of phosphorus excreted in the feces of a normal puppy is relatively small, while in rickets the quantity lost in this manner is tremendously increased. Experimentally, we have been able to so regulate the amount of alkaline salt mixture added to our basal diet as to divert the phosphorus almost entirely from urine to feces. If a large excess of phosphorus free salt mixture is given, only a trace of phosphorus appears in the urine, the retention of this element may be increased in the body and healing occur. On the other hand, a smaller amount of the salt appears to produce the optimal degree of alkalinity for the activation of the rachitic process and severe rickets may result.

If rickets can result from too high a degree of alkalinity in the intestines of an individual on a well constituted diet, as is frequently the case of artificially fed infants, it is possible that this condition may be the result of insufficient HCl in the gastric secretion or an excess of alkali in the intestinal juices. Those infants who have relatively little HCl in the gastric juice may develop normally when breast fed, but if the diet is changed to foods having a higher potential alkalinity the amount of acid present may be insufficient for normal mineral metabolism. Since cow's milk is potentially alkaline in reaction and contains a large amount of buffer substance as compared with human milk, on the above hypothesis we can explain why infants fed on cow's milk are more prone to rickets than those who are breast fed. Individual differences in HCl secretion may also explain why one of a pair of breast fed twins may be rachitic and the other not. Furthermore, the fact that inanition greatly retards the rachitic process can also be explained on this hypothesis since the products of katabolism of body tissue are acid in reaction and may help to restore the normal acid-base balance.

While we do not consider our experiments sufficiently numerous, and the many factors involved in so complex a salt mixture as we have used well enough controlled to permit us to draw final conclusions, they have suggested lines for further study. Experiments cited above, supplemented by others now under way, will be published in detail at a later date.

102 (2334)

Anaphylactic reactions in isolated rabbit lungs.

By W. H. MANWARING, H. D. MARINO and A. C. BEATTIE.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, Calif.*]

It was shown by Coca[1] that the dominant physological reaction in acute anapylaxis in rabbits is an occlusion of the pulmonary blood vessels with red blood corpuscle masses. Coca has evidence that this conclusion is not due to thrombosis or hemagglutination (embolism). He therefore concludes that it is due to an acute pulmonary vasoconstriction, comparable with the acute bronchoconstriction in anaphylactic guinea pigs. We have endeavored to test this conclusion by our routine perfusion technique.

On perfusing the isolated lungs of horse serum sensitized guinea-pigs with 2 per cent horse serum in Locke's solution, a distinct bronchoconstriction is demonstrable within thirty seconds, increasing to a complete bronchial stenosis by the end of two minutes.[2] At this stage of the reaction, the lungs can neither be expanded nor collapsed by changes in air pressure in the tracheal canula.

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[1] Coca, A. F., The Mechanism of the Anaphylaxis Reactions in the Rabbit. *J. Immunol.*, 1919, iv, 219.

[2] Manwaring, W. H., and Kusama, Y., Analysis of the Anaphylactic and Immune Reactions by Means of the Isolated Guinea-pig Lungs. *J. Immunol.*, 1917, ii, 157.

If the isolated lungs of rabbits sensitized to horse serum by Auer's method are similarly perfused, no bronchoconstriction is demonstrable. A slight increase in perfusion resistance takes place, the rate of perfusion flow being reduced about 8 per cent. This increase, however, is so slight that it cannot be assumed to play a clinically recognizable rôle.

That a marked vasoconstriction, comparable with the acute bronchoconstriction in anaphylactic guinea-pigs, is physiologically possible in isolated rabbit lungs, is shown by the 95 per cent reduction in perfusion flow with histamine and the 75 per cent reduction with Vaughan's protein split product.[3]

Perfusion tests have not yet been made with rabbits sensitized by Coca's method. If equally negative results are obtained with these more highly sensitized rabbits, one must conclude, either: (1) that a preliminary reaction in the circulating blood (anaphylotoxin formation) is necessary for the pulmonary vasoconstriction described by Coca, or (2) that the site of the vascular occlusion is in some part of the pulmonary circulation other than the branches of the pulmonary artery.

We believe that the pulmonary capillaries will ultimately be shown to be the essential site of this occlusion. If this proves to be the case, the occlusion of the pulmonary blood vessels with red blood corpuscle masses will not be due to smooth-muscle reactions (vasoconstriction), as currently assumed, but to increased capillary blood viscosity secondary to a suddenly increased capillary permeability. This view is in accord with recent studies in capillary permeability in canine anaphylaxis.[4, 5, 6, 7]

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[3] Manwaring, W. H., and Marino, H. D., Serological Reactions in Isolated Rabbit Lungs, I. Reactions to Histamine and to Vaughn's Split Product. *J. Immunol.*, 1923, viii, 317.

[4] Manwaring, W. H., Chilcote, R. C., and Hosepian, V. M. Capillary Permeability in Anaphylaxis. *J. Am. Med. Assn.*, 1923, lxxx, 303.

[5] Petersen, W. F., and Levison, S. A., Studies in Endothelial Permeability. II. The role of the endothelium in canine anaphylactic shock. *J. Immunol.*, 1923, viii, 349.

[6] Petersen, W. F. Jaffe, R. H., Levison, S. A., and Hughes, T. P., Studies in Endothelial Permeability. IV. The modification of canine anaphylactic shock by means of endothelial blockade. *J. Immunol.*, 1923, viii, 367.

[7] Manwaring, W. H., Hosepian, V. M., and Thomson, W. L., Quantitative Study of Anaphylactic Capillary Permeability. *J. Am. Med. Assn.*, (In press.)

ABSTRACTS OF COMMUNICATIONS.

Pacific Coast Branch.

Thirty-ninth meeting.

San Francisco, California, December 12, 1923.

103 (2335)

The production of the adiposogenital syndrome in the rat, with preliminary notes upon the effects of a replacement therapy.

By PHILIP E. SMITH, A. T. WALKER, and JAMES B. GRAESER.

[*From the Department of Anatomy, University of California, Berkeley, Calif.*]

The answer to the question whether the genital atrophy, skeletal undergrowth, and excessive adiposity of the adiposogenital syndrome are due to a pituitary deficiency or to an injury of the hypothalamus without pituitary involvement has rested heretofore upon observation made in two closely allied ways, (1) the extent of the injury inflicted as determined at the time of operation, (2) the extent of the injury as determined by autopsy and a study of sections. Both of these methods are open to the objection that the most careful study may fail to reveal the extent of damage done.

Another method is available for determining the significance of the pituitary and brain injury in the genesis of this syndrome, namely, that of a replacement therapy. If we have any confidence in the functional specificity of the endocrine glands, it follows that we must admit the significance of a pituitary impairment as the causative factor if the adiposogenital syndrome or any of its components can be corrected by pituitary administration. This point has been previously discussed (Smith and Smith) in connection with reports upon a replacement therapy in the hypophysectomized tadpole, in which it was shown that the enlargement of the fat organ, and the pigmentary, growth, and endocrine disturbances which invariably follow hypophysectomy in the tadpole, can be corrected by pituitary administration. It is our intention to use this method to make an analysis of the part played by the pituitary in the adiposogenital syndrome of the mammal.

A considerable number of rats (seven) which display Frölich's syndrome together with several others which display an upset in the reproductive mechanism have been secured by a rather simple procedure. By removing one parietal bone together with a part of the caudal and ventral walls of the orbit a good view is secured of the stalk of the pituitary. A fine hypodermic needle is then inserted into the gland and 0.01 to 0.013 cc. of a 10 per cent chromic acid solution injected with a syringe fitted with an attachment by means of which amounts as small as 1/600 cc. can be accurately injected. The spread of the injected fluid through the gland appears to be aided by the rich vascular network, while injury to adjoining structures is hindered by the capsule.

An experiment upon one animal has been finished to date. This animal, a female, operated upon February 17, failed to show the usual sex cycles, although displaying no adiposity. On May 22 one ovary and a part of the corresponding horn of the uterus were removed for diagnosis. These structures were found to be much smaller than normal. The ovary contained no corpora and no follicles approaching maturity. There was considerable interstitial tissue. After allowing a few days for recovery from the operation a daily injection of anterior hypophysial substance by the method of Evans and Long was begun on May 28. Ovulation as revealed by the vaginal smears took place on June 10 and June 14, the usual four day cycle thus being displayed. Autopsy on June 17 revealed a normal sized ovary with many corpora and large follicles. The uterus had become normal in size. No pituitary could be distinguished with the binocular. A study of serial sections revealed the presence of apparently normal neural and intermediate lobes but no typical anterior lobe cells.

It thus seems clear that in this case we had caused the ovaries to become structurally atrophic and functionally inactive by injuring the pituitary body, and that a repair was effected by the administration of anterior lobe substance, a result which it would seem removes in this case the possibility of a brain lesion being the causative factor except in so far as such a lesion might have directly contributed to the impairment of the hypophysis. This result we realize must be confirmed by other instances before generalizations can be ventured upon.

104 (2336)

The synthesis of hippuric acid by jaundiced animals.

By J. L. BRAKEFIELD and CARL L. A. SCHMIDT.

[*From the Department of Biochemistry and Pharmacology, University of California, Berkeley, Calif.*]

Experiments were carried out to determine the proportion of benzoic acid which dogs and rabbits whose common bile ducts had been ligated can conjugate with glycocoll to form hippuric acid. Two rabbits whose bile ducts had been ligated excreted only 40 per cent of the amount of hippuric acid which was synthesized by normal rabbits of the same weight, the remainder of the benzoic acid being eliminated uncombined. It was found that a normal dog which was maintained on a constant diet was able to conjugate 95 per cent of a dose of benzoic acid but after ligation of the bile duct the amount of hippuric acid which was synthesized dropped to about 60 per cent. A similar result was obtained with another jaundiced dog. The results are contrary to the work of Kühne,[1] who found no hippuric acid synthesized by a jaundiced dog, but are essentially in accord with those of Folwarczny[2] and others[3] who studied human cases, and the experiment of Lewis[4] on a rabbit. The results of this and other work on jaundice will be published in detail in the near future.

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[1] Kühne, W., *Virchow's Arch.*, 1858, xiv, 310.

[2] Folwarczny, *Z. Kais. König. Gesellschaft der Aertzte zu Wien*, 1859, xv, 225.

[3] Neukomm, J., *Klin. der Leberkrankh.*, 1861,ii, 537. Schultzen, *Dubois Arch.*, 1868, 719. Baumstark, F., *Ber. klin. Woch.*, 1873, xliii, 41. These references are quoted from C. von Noorden, ''The Pathology of Metabolism,'' Chicago, 1907, 268.

[4] Lewis, H. B., *J. Biol. Chem.*, 1921, xlvi, 73.

105 (2337)

Recovery from the hyperpnea of moderate exercise. The recovery ratio.

By A. W. HEWLETT and J. R. NAKADA.

[*From the Department of Medicine, Stanford University Medical School, San Francisco, Calif.*]

After muscular exercise, the excessive pulmonary ventilation lessens rapidly at first and then more slowly. The promptness of recovery is obviously one of the factors which indicate the individual's reaction to the exercise. It is difficult to measure the total duration of hyperpnea because the final approach to the resting level is very gradual and there is no sharp end point. We have therefore sought a numerical expression for the earlier and more rapid stages of recovery.

The test exercise here reported consisted of 200 hops at a rate of 120 hops per minute, or of climbing 200 steps on a treadmill at a rate of about 80 per minute. The rate of pulmonary ventilation was recorded just before and for several minutes just after the exercise, using the recorder described by Slonaker.[1] We found that in these experiments, the rate of pulmonary ventilation, at any moment, t, after the exercise stopped, roughly approximated a logarithmic curve of the form

$$h.\ r^t + c$$

According to such a formula the ventilation for successive minutes after the exercise stopped may be expressed as follows:

For the first minute	$M \quad + c$
For the second minute	$M\ r \ + c$
For the third minute	$M\ r^2 + c$
For the fourth minute	$M\ r^3 + c$

and so on. In our calculations, c equalled the rate of ventilation before the exercise started, M was determined by subtracting c from the ventilation of the first minute and r was so chosen as best to fit the ventilation of subsequent minutes. Since r is a fraction the value of the expression diminishes with each succeeding minute and the ventilation ultimately approaches c, the resting ventilation. The rapidity of the approach depends upon the value of r. For this reason r may we called the recovery ratio.

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[1] Slonaker, J. R., *Sci.*, 1923, lvii, 180.

In the accompanying tables a comparison is made between the observed ventilations and those calculated by this formula. In each case, averages of a number of experiments were used so as to lessen the accidental fluctuations in ventilation, which are sometimes rather marked. It will be noted that observed ventilations roughly correspond to the postulated type of curve. Differences in the speeds of recovery are indicated by the volumes of r.

TABLE I.

Comparison of observed and caluculated values of recovery from the hyperpnea induced by muscular exercise. In each case the averages of observed values are first given, next the calculated values and finally the differences between the two.

Subject	Exercise	No. of observations	Minute volumes before exercise	Minute volumes on successive minutes after exercise.				M	R
				First	Second	Third	Fourth		
M. G.	200 hops	6	6.5	22.8 22.8	16.2 15.7 —.5	12.0 11.7 —.3	8.7 9.4 +.7	16.3	.565
M. G.	200 hops	6	7.1	26.0 26.0	18.8 18.3 —.5	13.5 13.8 +.3	11.0 11.1 +.1	18.9	.594
A. W. H.	200 steps	20	7.4	26.7 26.7	14.6 15.0 +.4	10.2 10.4 +.2	9.2 8.6 —.6	19.3	.394
J. S.	200 steps	7	9.7	27.5 27.5	17.6 18.4 +.8	13.6 13.9 +.3	12.7 11.7 —1.0	17.8	.489

106 (2338)

Some comparisons of rotatory power and reducing power of ultrafiltrates of blood plasma.

By G. L. FOSTER.

[*From the Department of Biochemistry and Pharmacology, University of California, Berkeley, Calif.*]

Comparisons have been made of the rotatory power and copper reducing power of ultrafiltrates from normal beef and rabbit plasma. The technique of ultrafiltration was essentially that of Marshall and Vickers.[1] The filtrates, without further treatment, were observed in a Schmidt-Haensch triple field polariscope with scale graduated to 0.01 degree. The first readings were made within four or five hours after drawing the blood. The readings were repeated on succeeding days. The copper reducing power was determined by the method of Shaffer and Hartmann. The reducing power remained constant for several days. Bacterial action was prevented by addition of toluene.

The experiments are divided into three groups:

1. The plasma was filtered directly with no preliminary treatment.
2. The plasma was acidified with eight drops of glacial acetic acid per 100 cc. before filtration.
3. The plasma was partially precipitated with small volumes of 20 per cent sodium tungstate and 1.3 N sulfuric acid before filtration.

The results of a few typical experiments are shown in Table I. The sixth column of the table shows the rotation calculated from the reducing power assuming the specific rotation + 52.5°.

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[1] Marshall, E. K., and Vickers, J. L., *Bull. Johns Hopkins Hosp.*, 1923, xxxiv, 1.

TABLE I.

Sample (plasma)	Rotation				
	Observed (degrees)				Calculated (degrees)
	1st day	2nd day	3rd day	4th day	
Beef	0.10	0.11	0.12	0.11	0.11
Beef	0.10	0.08	0.08		0.10
Beef	0.15	0.15	0.16		0.16
Rabbit	0.13	0.13	0.14		0.14
Rabbit	0.12	0.13	0.12	0.12	0.16
Beef (acidified)	0.14	0.15	0.14		0.16
Beef (acidified)	0.11	0.10	0.11		0.12
Rabbit (acidified)	0.15	0.14	0.16		0.17
Beef: 35 cc. plasma 5 cc. 20 per cent Na_2WoO_4 5 cc. 1.3 N H_2SO_4	0.12	0.14	0.13		0.17
Rabbit: 25 cc. plasma 3 cc. 20 per cent Na_2WoO_4 3 cc. 1.3 N H_2SO_4	0.10	0.09	0.10		0.13

In no case have we observed any appreciable change from the initial rotation, and in general there has been substantial agreement between the observed and calculated rotations.

When we consider the extreme instability of gamma glucose,[2] it seems unlikely that its presence in normal blood can be detected by such experiments as these or by those of Winter and Smith.[3] The development of theories of carbohydrate metabolism based on ethylene oxide blood sugar would seem to be premature.

107 (2339)

Studies on the cholesterol and fatty acid content in the blood of normal and icteric infants.

By G. F. NORMAN (by invitation).

[*From the Department of Pediatrics of the University of California, San Francisco, Calif.*]

Cholesterol and fatty acid studies on blood were undertaken primarily to determine the relation of these substances, if any, to icterus neonatorum, and to establish average values for infants.

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[2] Irvine, J. C., Fyfe, A. W., and Hogg, T.P., *J. Chem. Soc.*, 1915, cvii, 524.

[3] Winter, L. B., and Smith, B. A., *J. Physiol.*, 1922, lvii, 100.

The method which was employed was that which has been described by Bloor.[1] About one hundred determinations were made on the blood of infants and of older children varying in age from one to four and one-half years. Table I shows the results of these determinations in a number of average cases.

TABLE I.
Icteric Infants

No. in series	Cholesterol per 100 cc. Saponification values	Cholesterol per 100 cc. Nonsaponification values	Fatty acids per 100 cc.
	mg.	mg.	mg.
4	59	....	385
8	55	....	313
42	44	78	......
46	50	63	290
49	49	74	310
53	63	194	333
56	72	181	333
58	53	108	310
Non-icteric Infants			
31	53	....	308
32	46	....	217
40	26	42	283
43	81	106	375
44	46	52	200
45	31	53	300
47	43	66	400
48	42	69	270
54	66	175	333
55	59	125	320
62	69	80	240
69	66	149	166
76	64	93	......
77	84	94	......
78	65	83	......

Non-saponification values were difficult to read in some cases due to the development of a brownish-green color probably caused by the presence of bile derivatives. It will be observed that there are no marked differences in the amounts of fatty acid and of cholesterol in the two series of infants, but the values for blood cholesterol are markedly lower than those which have been given for normal adults. (The effects of the alimentary lipemia persisting, as is usually stated, from 8 to 12 hours, were obviated as far as possible by obtaining blood specimens before feeding. Cholesterol values, however, would probably be very little influenced.—Bloor, Studies on Blood Fat, II.)

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[1] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, lii, 191.

Table II shows a few values which were obtained on normal older children.

TABLE II.

No. in Series	Age	Cholesterol per 100 cc.		Fatty acids per 100 cc.
		Saponification values.	Nonsaponification values	
		mg.	mg.	mg.
74	4 Mo.	54	179	220
75	6 "	74	107	......
72	7 "	54	125	240
68	8 "	96	134	300
73	13 "	113	171	233
61	2 Yr.	151	191	330
63	2 "	110	217	275
51	4½ "	150	344	438

Cholesterol and fatty acid values were obtained in one case of severe anemia, first before transfusions of blood and then during the course of treatment. This infant showed marked improvement clinically, and the hemoglobin rose rapidly from 15 per cent to 68 per cent. The following are the results of the investigation:

Date	Cholesterol per 100 cc.		Fatty acids per 100 cc.
	Saponification values	Nonsaponification values.	
	mg.	mg.	mg.
March 23, 1922	9	17	111
April 25, 1922	87	100	240
May 2, 1922	117	169	390

The low cholesterol reading on the first examination in the saponified portion was rendered difficult due to the development of a brown discoloration, but did not exceed the value given.

SUMMARY AND CONCLUSIONS

1. Cholesterol or fatty acid content of the blood bears no relation to icterus neonatorum.

2. Low values for cholesterol seem quite constant in the blood of infants. This is in agreement with the results of previous workers reported elsewhere, although in the present in-

vestigation the figures which were obtained are somewhat lower than those which have been reported by others. A gradual rise to normal adult values is observed before the second year of life.

3. Adult fatty acid values obtain throughout infancy.

108 (2340)

The relationship of virulence of hemolytic streptococcus to heat resistance.

By T. D. BECKWITH and E. J. ROSE (by invitation).

[*From the Department of Bacteriology and Experimental Pathology, University of California, Berkeley, Calif.*]

The Department of Bacteriology and Experimental Pathology of the University of California possesses a strain of hemolytic streptococcus which has been maintained constantly for a period of years by culture upon blood agar in test tube and by passage *in vivo* through rabbits. Intrapleural injection is used and the organism has been stored in the pleural exudate produced. This fluid exudate between passages is kept at icebox temperature. Injections are made from the previous pleural fluid after three to five weeks. To date this *in vivo* strain has passed through sixty-five animals or "generations".

The virulence of the passage portion of this strain is such that 1/100,000 to 1/1,000,000 cc. of this fluid is lethal to a 3,500 gm. rabbit. On the other hand, one cubic centimeter of the portion which has been propagated in test tube culture does not induce death in an animal of similar weight. The numbers of organisms per cubic centimeter in each inoculated fluid are comparable.

An interesting difference in heat resistance has developed between these two portions of this strain of *S. hemolyticus.* Using exposure to heat in a water bath and with all conditions as nearly similar as are experimentally possible, including numbers of organisms in the inoculum, the diluting medium and with properly controlled thermometers it has been determined that the more virulent organisms are more susceptible to the lethal effects of heat than are those the virulence of which is decreased. This difference in thermal death point amounts to approximately five degrees centigrade with like exposure periods. This difference is the same whether the suspending medium be skimmed milk or 50 per cent rabbit serum in physiological saline. A more complete report will be given at a later date.

ABSTRACTS OF COMMUNICATIONS.

Minnesota Branch

Fourteenth meeting.

Minneapolis, Minnesota, December 12, 1923.

109 (2341)

A statistical study of the form and growth of Bacterium coli.

By ARTHUR T. HENRICI.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

In previous studies[1] of the form and growth of bacteria, the length of the cells has been taken as a measurement of size because apparently the width did not vary nearly so much, and because the width could not be determined accurately with the technique used. In the present study which has had for its purpose the establishment of the normal variations in size and form of *Bacterium coli* when grown on standard beef extract agar, a somewhat different technique was used. Photomicrographs were made from slides prepared by the negative staining method of Benians, and the photographic negatives were again projected so that the final magnification was 30,000 diameters. The projected image was traced on paper and from these tracings the length and area were determined. The growth curve was obtained by counting the cells by a technique which has been previously published.[2]

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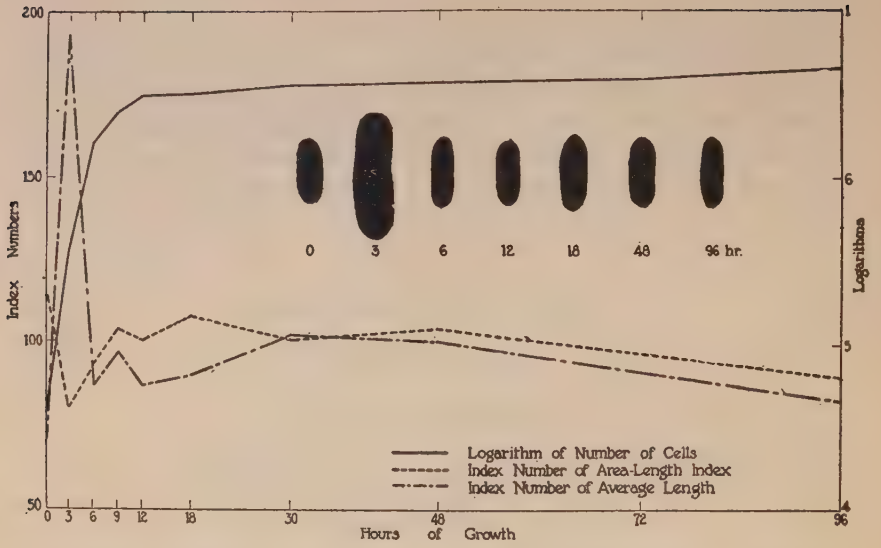
[1] Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 132; 1922, xx, 179.

[2] Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 293.



The results are shown in the accompanying graph. The diagrams of the cells have been traced from composite photographs of the tracings of the projected images and indicate roughly the average size and form of the cells at the various time periods. The rate of multiplication is indicated by the curve of the logarithm of the number of cells. Assuming that the cells are symmetrical about the axis, the area of the projected image divided by the length squared may serve as an index of variation in form, just as the weight-length index has been used for higher organisms. The cells were found to vary from spherical to cylindrical types, the area-length index being higher for the spherical forms. The average length of the cells and the average area-length index at each time period have been plotted as index numbers, the index in each case being the general average for the entire series. It will be seen that, like *Bacillus megatherium,* the cells show a marked increase in length followed by a rapid decrease to the original size during the logarithmic growth phase, and then show minor fluctuations. As the cells do not increase in thickness as much as they do in length, they become more cylindrical during the period of increased size, as indicated by a decrease of the area-length index. Frequency curves of the length of cells show marked increased range and dispersion during the period of increased length with some skewness at all periods. No tendency to bimodality was noticed. The frequency curves of the area-length index, in general, showed skewness in the opposite direction and decreased range during the period of increased size. It would seem then that there is throughout, a negative correlation between the length of the cells and the area-length index. The coefficient of variation for both length of cells and area-length index was increased during the period of increased size.

110 (2342)

On the growth of the human cerebellum in early life.

By RICHARD E. SCAMMON and HALBERT DUNN.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.*]

All investigators of the ponderal growth of the human cerebellum in early life have been impressed by the rapid rate of increment of this part of the brain in the first year. This has lead certain students of human growth to conclude that the rapid growth of the cerebellum in infancy is correlated with the marked development of muscular co-ordination and activity in this period.

The following figures represent an attempt to test the truth of this concept. They consist of the results derived from the computations of the growth in volume of the cerebellum in the fetal period and of weight in the period of infancy. These computations are based upon the following empirical formulae:

1. For the relation between the cerebellum volume and body-length in the fetal period:

Volume of the cerebellum (cc.) $= 0.01\,[(0.095 \text{ crown heel length in cm.})^{4.9} + 20.0]$, as developed by Dunn.[1]

2. For the relation between body-length and age in the fetal period:

$$\text{Age in fetal months} = 2.3 + \frac{2.5\,(\text{body-length in cm.})}{28} + \frac{(\text{body-length in cm.})^2}{784}$$

as developed by Scammon and Calkins.[2]

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[1] Dunn, H., *J. Comp. Neurol.*, 1921, xxxiii, 405.

[2] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 353.

3. For the relation between cerebellum weight and age in postnatal life:

$$\text{Weight of cerebellum (gm.)} = \frac{\text{Age (years)}}{0.094 + 0.0078 \text{ age (years)}} + 19.8,$$

as developed by Scammon and Dunn.[3]

Unfortunately it is necessary to use volumes for the fetal period and weights for infancy in these calculations, but the difference between the volume and the weight of the cerebellum is relatively small in prenatal life, and the correction for this slight difference would accentuate rather than reduce the changes herein recorded.

As will be seen from tables I and II, the absolute volume of the cerebellum increases at first slowly, and then rapidly to the close of the fetal period; the increment in the tenth month being 8.12 cc. After birth, on the other hand, the increment in weight of the cerebellum undergoes a constant decrease from 6.19 grams in the first postnatal month to 2.52 grams in the thirteenth month.[4]

The percentage increment in weight or volume also shows striking changes. This index is determined by dividing the absolute increment in each month by the value at the beginning of the month and multiplying by 100. The percentage increment showed a marked rise from the fifth to the sixth fetal month, the value being over 162.5 per cent in the latter interval. After the sixth fetal month the percentage increment decreases at first rapidly and then more slowly, falling to about 38.8 per cent for the first month after birth and to 3.34 per cent for the last month in the first year.

These results are shown in graphic form in the following figure where solid columns are used to represent the monthly percentage increments, and open columns the absolute monthly increments.

These figures indicate that while there is indeed a rapid growth in the cerebellum the first year, this growth is not stimulated by the factors of extra-uterine environment. This growth in early infancy is rather to be regarded as a diminishing residuum of the tremendous growth energy of the organ which is exhibited in the latter half of intra-uterine life.

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[3] Scammon, R. E., and Dunn, H. L., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 114.

[4] All calculations, both prenatal and postnatal, are made in lunar months of four weeks.

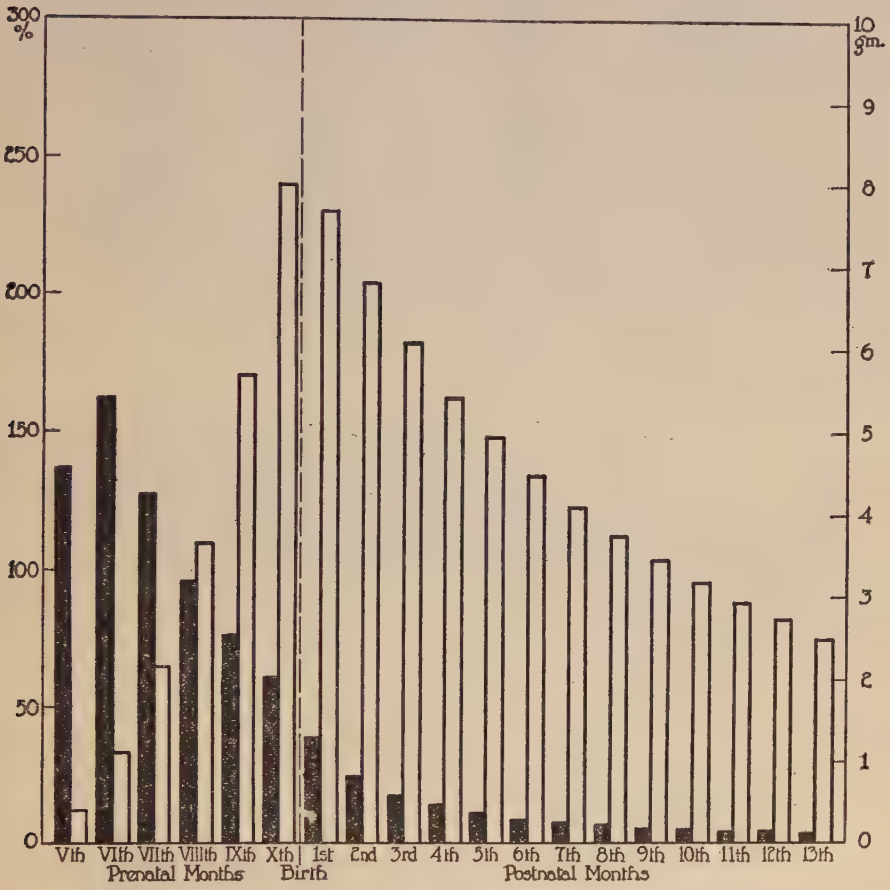

TABLE I.

Volume of the cerebellum in the fetal period, weight of the cerebellum in the first year.

Age fetal months (of four weeks)	Cerebellum volume (cc.)	Age postnatal months (of four weeks)	Cerebellum weight (gm.)
5 months	0.64	Birth	19.80
6 months	1.68	1 month	27.49
7 months	3.83	2 months	34.31
8 months	7.50	3 months	40.40
9 months	13.18	4 months	45.87
10 months	21.30	5 months	50.82
		6 months	55.30
		7 months	59.40
		8 months	63.14
		9 months	66.58
		10 months	69.75
		11 months	72.69
		12 months	75.41
		13 months	77.93

TABLE II.

Absolute and percentage monthly increments in the cerebellum volume and weight.

Age	Absolute monthly increments (vol. or wgt.)	Monthly percentage increments
	(cc.)	
5th fetal month	0.37	137.03
6th fetal month	1.04	162.50
7th fetal month	2.15	127.97
8th fetal month	3.67	95.82
9th fetal month	5.68	75.73
10th fetal month	8.12	61.60
	(gm.)	
1st postnatal month	7.69	38.83
2nd postnatal month	6.82	24.80
3rd postnatal month	6.09	17.75
4th postnatal month	5.47	13.54
5th postnatal month	4.95	10.79
6th postnatal month	4.48	8.82
7th postnatal month	4.10	7.41
8th postnatal month	3.74	6.30
9th postnatal month	3.44	5.45
10th postnatal month	3.17	4.76
11th postnatal month	2.94	4.22
12th postnatal month	2.72	3.74
13th postnatal month	2.52	3.34

It may be urged that the intra-uterine growth of the cerebellum may represent a response to the muscular activity indicated by quickening, which usually takes place some time in the fifth month when the cerebellum begins its more active growth. This argument, however, seems invalidated by the fact that all of the parts of the brain, as well as all other of the portions of the body show an increased growth in weight at this time, and therefore there is no particular reason for assuming that the growth of the cerebellum at this period is activated by any special factor which is not influencing the other parts of the body.

111 (2343)

Carotin in Perillus bioculatus (Fab.) and its derivation from the lymph of Leptinotarsa decemlineata (Say).

By L. S. PALMER and HARRY H. KNIGHT.

[*From the Sections of Dairy Chemistry and Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

One of us[1] has shown that the variations in the hypodermal color pattern of the stink-bug *Perillus bioculatus* (Fab.) from white and black to red and black (with various intermediate yellow and black forms) are not caused by inheritance but by variations in physiological activity, which can be controlled by the temperature of the environment, and influenced by sexual functions, such as egg laying. The food of the stink-bug is always highly colored with orange-yellow pigment for it consists almost exclusively of the eggs and larvae of the potato beetle, *Leptinotarsa decemlineata* (Say), as well as the adult beetle itself. When the larvae and adult beetles are attacked the golden yellow lymph is the only portion eaten.

A chemical examination of the pigment in the lymph of the potato beetle showed that it consists exclusively of carotin. No other carotinoids could be detected. The concentration of carotin in fresh lymph obtained from 200 full-grown larvae was found to be 0.0136 per cent, which is as high as is encountered in fresh green leaves.

The red and yellow colors in the hypodermis of the stink-bug were also found to be due to carotin. Some water-soluble substance yielding yellow solutions was also present in the hypodermis, but it is doubtful whether it contributed greatly to the color pattern.

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[1] Knight, H. H., *19th Report, State Entomologist of Minnesota,* 1922, 50.

With the exception of the early work of Physalix[2] on the red pigment in the bug *Phyrrhocous apterus* L., this is the first definite identification of carotin in Hemiptera and establishes for the first time the biological origin of carotin in an insect in a manner analogous to the identification of the source of carotin in cattle in an earlier experiment by one of us.[3]

112 (2344)

Anthocyanin and flavone-like pigments in phytophagous and predaceous forms of Hemiptera.

By L. S. PALMER and H. H. KNIGHT.

[*From the Sections of Dairy Chemistry and Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

The identification of carotin as the chief cause of the red and yellow hypodermal colors seen in the predaceous stink-bug *Perillus bioculatus* (Fab.), reported in the preceding abstract, suggested an examination of the red pigment of other Hemiptera. It was found that red pigment in phytophagous and predaceous families of this order of insects is not limited to one type of substance. Water-soluble pigments appear to be more common than carotin. The aphid (*Tritogenaphis rudbeckiae,* Fitch) owes its vermilion color chiefly to an anthocyanin-like pigment, although small quantities of carotin also occur in the bug. On the other hand the red color of the red and black patterned phytophagous box-elder plant-bug (*Leptocoris trivitatus,* Say), the milk-weed plant-bug (*Lygaeus kalmii,* Stal), the bladder-nut plant-bug (*Lopidea staphyleae sanguinea,* Kngt.), the maple plant-bug (*Coccobaphes sanguinareus,* Uhler), and the predaceous Assassin-bug (*Eulyes illustris,* Stal) is due to a flavone-like pigment.

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[2] Physalix, C., *Compt. rend.*, 1894, cxviii, 1282.

[3] Palmer, L. S., and Eckles, C. H., *J. Biol. Chem.*, 1914, xvii, 191.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

One hundred and thirty-seventh meeting.

Presbyterian Hospital, February 20, 1924.
President Jackson in the chair.

113 (2345)

Intarvin in phlorhizinized dogs.

By ETHEL M. BENEDICT and RANDOLPH WEST.

[*From the Department of Medicine, Presbyterian Hospital, Columbia University, New York City.*]

These experiments were undertaken to determine whether intarvin—glyceryl margaric ester—yields extra sugar in the completely phlorhizinized dog. Ringer[1] has shown that propionic acid is quantitatively converted into dextrose in such dogs, and if the β oxidation of saturated normal fatty acids holds, one might expect each molecule of margaric acid to yield one molecule of propionic acid during oxidation in the body. In the phlorhizinized animal this should appear as sugar. 26.8 gm. of intarvin, if pure, could yield 10 gm. of dextrose, assuming that one molecule of the fat would yield one molecule of glycerol and three molecules of propionic acid, and that the glycerol and propionic acid could yield two molecules of dextrose. This calculation takes into consideration the 12 per cent mineral oil added to intarvin but does not allow for the fact that the fat probably is a mixture of about 85 per cent margaric acid and 15 per cent stearic acids combined with glycerol.

Phlorhizin was administered either in olive oil twice a day, or in 1.2 per cent Na_2CO_3 every eight hours, subcutaneously. Dogs were fasted for three days before phlorhizination, and urine discarded until a constant D:N ratio was obtained for a 24 hour control period, then the animal was fed intarvin or butter mixed with lean meat hash and the urine followed for the following 24 hours or until the D:N ratio returned to the figure obtained for the control period. Toluene or NaF (1 per cent) were the preservatives used, nitrogen was determined by Kjeldahl's method, sugar by the Allihn method, total acetone bodies by the VanSlyke method, and organic acids by the method of Van Slyke and Palmer. The dogs were most irregular in acetone body formation, and the results in this field are not felt to be of much value.

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[1] Ringer, A. I., *J. Biol. Chem.*, 1912, xii, 511.

The results of attempts to introduce intarvin by stomach tube were discarded as it was found that some dogs gave "extra sugar" and marked rises in D:N ratio on merely introducing water by stomach tube, a process accompanied by some struggling. Attempts to use phlorhizinized dogs depleted of glycogen by epinephrin were also discarded, as such dogs gave D:N ratios of about 3.0 and in one such animal 10.0 gm. dextrose subcutaneously yielded but 5.5 gm. extra sugar in the urine. One dog yielded about 5 gm. extra sugar following 55 gm. intarvin and 200 gm. lean meat hash eaten, but this dog when fed the same hash and 55 gm. butter yielded about the same amount of extra sugar, so the extra sugar could not be interpreted as coming from intarvin. Before interpreting extra sugar as coming from intarvin the intarvin and meat must be eaten, not given by stomach tube, must be absorbed from the intestine, and the same dog must not give extra sugar on eating butter and meat. There must be a D:N ratio of about 3.70, as the epinephrinized dogs may not excrete sugar quantitatively, while in a dog with control period ratio of 3.59, 10.0 gm. dextrose subcutaneously yielded 9.64 gm. extra sugar—a fact long known.

Bearing these points in mind we report the results on two dogs which fulfill the above requirements. We have not been able to demonstrate extra sugar, nor any rise in acetone bodies nor in organic acids in the urine, but these last facts mean little as butter also failed to give the expected rise in acetone. Although we know that the intarvin disappeared from the intestine, we cannot be certain whether it was burned or stored, though the former seems probable in a phlorhizinized dog on the sixth day of a fast. It is a possibility that larger amounts of intarvin fed, and urinary studies extended for more than 48 hours after the feedings might show extra sugar. Such experiments are being carried out.

We are indebted to Prof. Ralph McKee for a supply of glyceryl margaric ester without mineral oil, for margaric acid, and for intarvin.

Dog 6. Male. 9.5 Kg. Last food November 16, 1923. Phlorhizin in olive oil. Toluene Preservative.

Date 1923	Hour	Phlor-hizin	Urine Volume	N	Urine Dextrose	D:N Ratio	Total acetone	Remarks.
Nov.		gm.	cc.	gm. per l.	gm. per l.		gm.	
19	5 p. m.	0.5						
20	9 a. m.	1.0						
	5 p. m.	1.0						
21	9 a. m.	1.0						
	1 p. m.							Dog voided. Discarded.
	5 p. m.	1.0						
22	9:30 a. m.	1.0	662	9.42	33.8	3.59	0.397	Catheterized.
	1:10 p. m.		180	8.55	32.5	3.80	0.103	Catheterized.
	1:50							Dog ate 32 gm. glyceryl Margaric ester and 100 gm. lean meat hash.
	5:00	1.0						
23	9:30 a. m.	1.0	652	13.3	48.5	3.64	0.280	Catheterized.
	1:30 p. m.		150	8.59	29.3	3.41	0.274	Catheterized. A little vomitus in urine, no visible fat.

1:45 p. m. Dog killed. Gastro-intestinal tract below diaphragm dissected out, all contents removed and found to contain 1.9 gm. fat. No stools during experiment.

Dog 8. Male. 13.6 Kg. Last food December 31, 1923. Phlorhizin in olive oil. Urine preservative NaF, final conc. 1 per cent.

Date 1923	Hour	Phlor-hizin	Urine Volume	N	Dextrose	D:N Ratio	Acetone bodies	Organic acids cc.0.1 N HCl	Remarks
Dec.		gm.	cc.	gm. per l.	gm. per l.		gm.		
2	5 p. m.	1.0							
3	9 a m.	1.0							
	5 p. m.	1.0							
4	10 a. m.	1.0							Catheterized. Discarded
	12:30 p. m.								
	5	1.0							Catheterized.
5	9 a. m.	1.0	978	10.0	39.7	3.97	0.427	1392	Catheterized. Dog ate 54 gm. glyceryl Margaric ester and 200 gm. lean meat hash.
	12:30 p. m.		160	9.43	34.9	3.71	0.139	184	
	4:40 p. m.	1.0							
	9 a. m.	1.0	1710	8.56	33.8	3.94	0.049	780	
6	12:30 p. m.		250	5.42	21.9	4.05	0.021	330	Catheterized. Dog ate 55 gm. butter and 200 gm. same hash.
7	9 a. m.	1.0	1730	8.04	30.8	3.83	0.085	802	
	12:30		275	5.42	21.2	3.91	0.050	434	

114 (2346)

Attempts to separate the active constitutent of ragweed pollen.

By LOUIS BAUMAN, MILDRED CHUDNOFF and GEORGE M. MACKENZIE.

[*From the Department of Medicine of Columbia University and the Presbyterian Hospital, New York City.*]

As ragweed appears to be the chief cause of hay fever in this country it seemed desirable to attempt to separate the substance in the pollen which is responsible for the sensitization of hay-fever patients.

A number of the constituents have been characterized by Heyl, but this author did not attempt to test systematically the various fractions which he isolated. Recently Coca and assistants subjected ragweed extract to the action of trypsin. On dialysis most of the nitrogenous consituents diffused through the membrane while the residue retained its activity; therefore the authors conclude that the active substance is not a protein.

Having found that 3 per cent ammonia was a useful solvent for the preparation of horse dander and other proteins used in clinical work, we also employed it for the extraction of ragweed pollen. Ammonia possesses certain advantages. It is a weak alkali with little racemizing or hydrolyzing effect and the protein obtained by precipitation of the ammonia extract with acetone is readily freed from alkali by drying *in vacuo* over sulfuric acid.

As the thick hard capsule is said to form 65 per cent of the pollen (Heyl) it was thought that extraction would have to be preceded by thorough grinding, but it was found that ammonia penetrated the capsule and completely extracted the active substance.

The preparations were tested by the cutaneous reactions on patients sensitive to ragweed. The varying sensitiveness of patients had to be considered in the interpretation of reactions.

The following results were obtained with the ammonia extract:

	Nature of the material	*Reaction*
1.	Pollen residue after 3 ammonia extractions	None
2.	Precipitate on adding acetone to ammonia extract	Strong
3.	Filtrate from 2	None
4.	Precipitate on saturating ammonia extract with ammonium sulfate dialyzed	Moderate

5.	Filtrate from 4	None
6.	Precipitate on half saturation with ammonium sulfate "globulin fraction"	Moderate
7.	Filtrate from 6	Very slight
8.	Precipitate on 1/4 saturation with ammonium sulfate	Moderate
9.	Filtrate from 8	Very slight
10.	Ammonium sulfate precipitate redissolved and twice reprecipitated with ammonium sulfate	Strong
11.	Ammonia extract dialyzed against 5 per cent sodium chloride solution for three days	Strong
12.	"Globulin fraction" undialyzed	Strong
13.	"Globulin fraction" dialyzed	Strong
14.	"Globulin fraction" dialysate	None
15.	"Albumin fraction" undialyzed	Slight
16.	"Albumin fraction" dialyzed	Slight
17.	"Albumin fraction" dialysate	Slight

COMMENT.

It would appear that the active substance can be completely extracted from ragweed pollen with 3 per cent ammonia and that it can be quantitatively removed from the ammonia extract by precipitation with acetone. As prolonged dialysis of the ammonia extract against 5 per cent sodium chloride fails to remove the active substance it is to be inferred that it is in the form of a large molecule or aggregate.

The "globulin fraction" seems to be the more active even when dialyzed for a number of days in collodion sacs and it is to be hoped that more data may be obtained by a study of the behavior of this fraction.

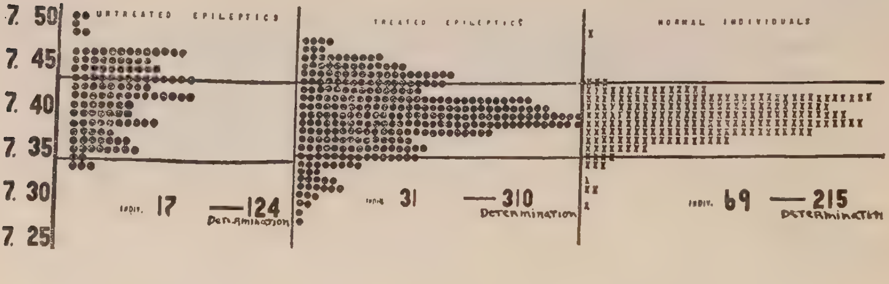
115 **(2347)**

The reaction of the blood in epilepsy.

By H. RAWLE GEYELIN, E. J. BIGWOOD and MARJORIE A. WHEATLEY.

[*From the Laboratory of the Presbyterian Hospital, Department of Medicine, Columbia University, New York City.*]

The hydrogen ion concentration of the blood in epilepsy has been determined in thirty-eight cases. In order to be able to ascertain the significance of any changes found, we have also studied the hydrogen ion concentration in the blood of twenty-eight normal individuals. In conjunction with the work of Hastings and Cullen, who have also made a certain number of observations on the pH of normal individuals, we find that the range for the latter is from 7.36 to 7.43. In contrast to this we find that the blood of treated and untreated epileptics ranges from 7.25 to 7.50. The blood of any given case of epilepsy shows a wider range of blood reaction than does the blood of a given normal individual. Chart No. 1 illustrates the extent of the range of blood pH determination in all the cases of epilepsy studied as contrasted with the range of variation observed in all the normal individuals studied. One case of hysteria, three cases of migraine and three cases of Jacksonian epilepsy, and one unclassified case show a range of blood hydrogen ion concentration which is similar to the range observed in normal people.



116 (2348)

The absence of both kidneys associated with hereditary abnormalities in mice.*

By HALSEY J. BAGG.

[*From the Memorial Hospital and Cornell University Medical College, New York City.*]

Recently the writer recorded the occurrence of fifty mice having in each case a solitary kidney.[1] They constituted 16 2/3 per cent of a group of 300 autopsied animals, descendants of animals that were subjected to light doses of x-ray irradiation. The treatment was apparently associated with the production of hereditary abnormalities of the eyes, feet and other parts of the body.

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* The writer gratefully acknowledges his indebtedness to the Carnegie Institution of Washington for aid in the care and breeding of a number of these animals.

[1] Bagg, H. J., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 146.

Several matings have now been made where in each case both parents had but one kidney, and the number of young per litter was considerably less than the average for animals with normal kidneys. Several of the young animals died during the first week of life and were promptly destroyed by their mother. The examination of a single litter of seven mice apparently throws considerable light on the high mortality shown in such animals and is considered of sufficient interest to warrant a brief report. In this instance, however, the male parent had but one kidney and was blind in the right eye, while the mother, although apparently normal, was heterozygous for such defects.

Two of the young of the above parents were found dead about 24 hours after birth. They had but recently died and at autopsy apparently showed complete absence of both kidneys. No other gross defects were noted except that one of the animals had a defective left eye. The remaining five animals were active and apparently in a good state of nutrition. Four of these were males with apparently no external deformities, and one was a female with a defective left eye. Three of the animals were killed about 24 hours after birth, and the point of interest is that the female with the defective left eye showed bilateral absence of kidneys and one male had only a very small remnant of renal tissue on one side of the body. One male had normal kidneys. The two remaining males of the litter were allowed to live and when examined two weeks later were found to be normal. No other visceral changes were noted in the animals and both adrenals were present in each case.

The above data apparently are very suggestive in accounting for the death of the young animals (with hereditary kidney abnormalities) during the early post-natal period. They show that in mice pre-natal development and at least several hours of post-natal life may continue despite the complete absence of both kidneys. Observations from similar matings may further show the average length of post-natal life compatible with such abnormal conditions of the viscera.

117 (2349)

A preliminary survey of the effects of ultra violet light on normal rabbits.

By J. H. B. GRANT and FREDERICK L. GATES.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

With no prejudice regarding the results to be obtained we have exposed five series of normal rabbits to the quartz mercury arc lamp daily for periods of three to seven weeks and observed at autopsy the weights of certain endocrine glands in relation to the body weights of the animals. It was expected that this survey might orient us for further studies in the biophysics of ultra violet light. The survey was made possible by the cordial cooperation of Doctors Brown, Pearce, and Van Allen, who placed at our disposal unpublished figures from similar examinations on a large number of normal rabbits covering a period of two years. Many controls are required because of individual and especially seasonal variations in the gland/body weight ratio.

Light colored or albino male rabbits, with ears shaved and backs clipped, in groups of eight (two controls), were exposed to the unfiltered radiations of a quartz mercury arc lamp for 30 minutes daily at a distance of one meter. This arbitrary dosage was probably too large at first and relatively ineffective during the final periods, when pigmentation had occurred. It caused a marked erythema with some desquamation, followed by moderate pigmentation. At the expiration of the chosen periods the rabbits were etherized and bled from the aorta. The following glands were weighed and examined in gross and microscopically: pineal, hypophysis, external parathyroids, thyroid, thymus, adrenals, and testicles. The weights of these glands were recorded in milligrams per kilogram net body weight after removel of the gastro-intestinal tract and its contained food and feces, and the expression of bladder urine.

The changes in weight, per cent, compared with the figures for 15 to 36 normal control rabbits examined at the same season of the year are shown in the accompanying table.

Effect of Daily Exposure of Normal Rabbits to Ultra Violet Light on the Weights of Endocrine Tissues.
Percentage change from normal. Weights in mg. per kilo of rabbit's net wgt.

Glands	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
Pineal	+14.0	— 1.4	+13.8	— 2.4	+ 7.0
Hypophysis	+25.0	+20.0	+23.0	+ 5.0	+ 6.0
Parathyroids	+34.6	+51.1	+31.9	+27.7	+14.1
Thyroid	—17.3	—19.5	— 2.4	+13.0	+12.0
Thymus	+13.0	+ 5.4	+ 9.3	—13.3	+14.7
Adrenals	— 3.0	+ 3.0	+17.0	+32.0	+13.5
Testicles	+ 7.0	— 6.5	+22.0	+11.0	+18.0

The findings for the thymus and the testicles may have no significance. The changes are not progressive, and individual variations are so wide in normal rabbits, due to age and other factors, as to include the limit figures of our experiment. The weight of the pineal gland also fluctuated above and below normal during the experimental period. At this season of the year—November to January—the hypophysis normally is decreasing in size. The rayed rabbits also showed a progressive decrease in the size of the hypophysis, but this decrease was delayed in the earlier weeks of the experiment so that the glands were 25 per cent heavier than in the controls. The thyroid and the adrenal glands showed parallel changes, namely, a relative loss in weight during the earlier weeks of exposure, followed by a rise above normal. We shall not discuss the possible significance of these changes at the present time.

Because of the relationship of the parathyroid glands to calcium metabolism on the one hand, and on the other hand the effect of ultra violet light on the absorption and excretion of calcium and the calcium level of the blood, interest centers on the effect of ultra violet light on the parathyroids. The changes in weight of these glands, relative to normal, are shown in the table and indicate a considerable hypertrophy of the parathyroids, especially during the earlier periods of the experiment. We are inclined to attribute the later decrease towards normal to the protection afforded the rabbits by pigmentation. The increases in weight were due to a true hyperplasia of the parenchymatous tissue, as shown by microscopic examination. When the figures for the third and fourth weeks were obtained, blood calcium determinations were made on the subsequent groups. While we have no normal figures for these particular rabbits, the average blood calcium concentrations for the last three groups at autopsy were 12.7; 12.9, and 12.6 mg. per 100 ml. of serum as compared with an average of 11.9 mg. for 60 normal control rabbits. Rothman and Callenberg[1] have reported rises above normal in the blood calcium level following light baths.

These observations have been substantiated by the results of a second experiment in which a group of rabbits was given increasing exposures of ultra violet light daily over a period of four weeks. This group showed a relative increase of 32 per cent in the parathyroid weight over the normal figures and the blood calcium concentration rose from 11.9 mg. per 100 ml. of serum before exposure to 12.2 mg. on the twelfth day, 13.5 mg. on the twenty-second day, and 12.9 mg. at autopsy.

These preliminary experiments have served to relate the effect of ultra violet light on calcium metabolism to the parathyroid glands. Whether the light acts primarily on the absorption and excretion of calcium and causes a rise in the blood calcium which results in an hypertrophy of the parathyroid glands, or primarily affects the glands themselves, remains to be determined.

118 (2350)

Feeding experiments on rats with plants at different stages of development. Part II.[1]

By BENJAMIN HARROW and FRANCIS KRASNOW.

[*From the Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York City.*]

This investigation was undertaken in order to ascertain the effect of supplementing a basal diet with dried samples of ungerminated, germinated and green corn. The object of the experiments was to see whether any growth-promoting substances were produced in the course of germination and "greening".

Experiments with ten groups of rats on diets: (1) regular (normal); (2) S + "A" + "B"; (3) S + "B"; (4) S + "A"; (5) S + "A" + ungerminated corn; (6) S + "B" + ungerminated corn; (7) S + "A" + germinated corn; (8) S + "B" + germinated corn; (9) S + "A" + green corn; (10) S + "B" + green corn—were carried out simultaneously under such conditions as to make the results, from a comparative standpoint, suggestive. During the first month each group of rats (four to a cage) receiving either ungerminated, germinated or green material, was given 4 gm. of such material per day; during the second month this was increased to 8 gm. and during the third month to 12 gm. The results at the end of the first month showed that aside from the group on the "regular" diet (1) the one showing the most notable increase in weight is group 10 (synthetic + "B" (yeast) + green). Since neither 6 (synthetic + "B" + ungerminated) nor 8 (synthetic + "B" + germinated) shows corresponding gains, the most obvious explanation is that a "growth" of vitamin (A?) or an increase in the quantity present, has taken place during "greening". This view would fall in line with suggestions made by several workers in the field (for example, Drummond[2] and Wilson[3]). But another explanation offers itself as a result of the following experiment:

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[1] Rothman and Callenberg, *Klin. Wchnschr*, 1923, ii, 1751.

[1] Harrow, B., and Krasnow, F., *J. Metabol. Research.*, 1922, ii, 401,

We carefully selected 100 seeds each of the ungerminated, germinated and seedling varieties and weighed them before and after air-drying, with the following results (weight in gm.):

	Fresh weight	Dry weight (room temp.)	Percentage dried material
Ungerminated	41.1	21.6	52.5
Germinated	46.2	24.1	52.1
Green	93.8	13.5	14.4

In our experiments, following the methods of other workers, we took the *same* quantities of the different varieties of *dried* material and compared results; whereas, it would seem, the more logical procedure would have been to take quantities in the proportion of 21.6, 24.1 and 13.5. In other words, instead of taking 4 g. ungerminated, 4 g. germinated and 4 g. green, we ought, perhaps, to take 4 ung., 4 × 24.1/21.6 germ (which is *about* the same) and 4 × 13.5/21.6 green. If this view be taken, then the rapid increase in weight noticed in Group 10 during the first month need not necessarily be interpreted as showing growth of vitamin; and the positive results claimed by other workers must be questioned. The results, of course, need not be interpreted as meaning that there is no growth of vitamin during germination and greening, but merely to suggest that the methods employed are open to criticism.

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[2] Drummond, J. C., *Biochem. J.*, 1921, xv, 530.

[3] Wilson, J. W., *J. Biol. Chem.*, 1922, li, 455.

Groups 5, 7 and 9 receiving a diet which included "A" (cod liver oil) and one form of corn but no "B" (yeast) began to gain markedly over 6, 8 and 10 on a diet including "B" and one form of corn but no "A" from the sixth week on. It would seem, therefore, fairly obvious that, comparatively speaking, the seeds (ungerminated, germinated and green) are richer in vitamin "B"; for in 5, 7 and 9 whatever "B" the rats get must come from the corn, just as in 6, 8 and 10 whatever "A" they get must come from the corn.

We are now engaged in comparing (a) effect of equal weights of dry material with (b) effect of the same number of seeds (ungerminated, germinated and green) and (c) effect of using weights obtained from the ratios

$$4 : \frac{4 \times 24.1}{21.6} : \frac{4 \times 13.5}{21.6}$$

119 (2351)

Sulfur metabolism. III.

By C. P. SHERWIN, A. R. ROSE and A. WEBER.

[*From the Chemical Research Laboratory, Fordham University, New York City.*]

The addition of cystine, cysteine or their derivatives to the control rations of rabbits caused a prompt and marked increase in the amount of sulfur eliminated in the urine. When these sulfur compounds were withdrawn the elimination of sulfur dropped to the control level, and in some instances even below this. This decrease in sulfur took place in the first two days of the period following the cystine sulfur feeding, and in most cases the first day. On the day following this marked depression of the sulfur elimination there was a distinct increase of sulfur in the urine. The cystine sulfur was not completely eliminated in the period during which it was fed, except in cases of phenyl acetyl benzyl cysteine. The increased sulfur output following the depression of sulfur elimination is considered as a lag. It would seem that the R-SH type of sulfur compound has some specific effect upon the mechanism of sulfur excretion. This effect is observed both with reduced sulfur and oxidized sulfur, but not with conjugated SO_4. In three cases, however, there was an increase in conjugated SO_4—namely 6 mg. for phenyl acetyl benzyl cystine and phenyl hydantoin cystine, and 10 mg. for acetyl benzyl cysteine. This "deferred lag" was unforeseen and must be taken into special account in our next series of this program. The excess elimination of sulfur in some of the tests may be charged to this "deferred lag"—but not altogether. In the case of phenyl acetyl benzyl cysteine the increased sulfur elimination exceeded the cystine sulfur added to the ration by one-half its amount. This could not come from any previously ingested R-SH added to the animal's normal ration. There seems to be positive evidence that ingestion of R-SH causes a sulfur elimination in addition to the sulfur immediately taken in so that the per cent of cystine sulfur as given in the table may include some endogenous reduced sulfur.

Phenyl hydantoin cystine was given to one rabbit in two test periods with an intervening control period of four days. In the first period there was a retention of the ingested sulfur amounting to 53 per cent of that administered, and in the second period there was an excess elimination amounting to more than four times the control value of the first day. In the four days between these two periods, and the days following the second period, there was an abrupt drop in urinary sulfur to a point below the normal. The total sulfur elimination for the two test periods and control days exceeds the total sulfur intake by 26 mg. If the depression in the control days were not considered it would be about three times this amount.

The elimination of these various compounds is summarized in the following table. The figures give that part of the sulfur which represents the cystine compounds. In cystine and cysteine most of the sulfur is eliminated in the oxidized form as noted in our last contribution. Blocking the -SH group of these with a benzyl, caused somewhat less oxidation to take place. This was also true when the amino group was blocked with acetyl and phenyl acetyl groups. When both the amino and sulfhydrate positions were blocked the oxidation was very much decreased, so that most of the cystine sulfur was excreted as of the type R-SH. It would seem from this table that in the oxidation of cystine the attack is most effective at the amino position and least (perhaps none) at the carboxyl.

SULFUR FROM CYSTINE COMPOUNDS.

	Fed	Eliminated			
		Total Cyst. S.		Total SO_4 S.	
	mg.	mg.	per cent	mg.	per cent
Cystine	321	297	93	264	89
Cysteine hydrochloride	258	265	102	207	78
Benzylcusteine	183	193	105	112	58
p-Cl Benzylcusteine	186	91	49	48	53
	186	174	94	111	64
Acetyl benzyl cysteine	153	181	118	105	58
Phenyl acetyl cystine	321	244	75	163	67
Phenyl acetyl benzylcysteine	117	185	158	60	32
Phenyl uramino cystine	315	277	88	114	41
Phenyl uramino cysteine	321	199	58	60	41
Phenyl uramino benzyl cysteine	123	45	36	(depressed below control level)	
Phenyl hydantoin cystine	345	168	47	81	41
	174	329	189	83	25
Phenyl hydantoin benzyl cysteine	125	82	65	0	0

120 (2352)

I. Experimental rabbit snuffles.

By L. T. WEBSTER (by invitation).

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

As part of a general experimental study of the epidemiology of certain bacterial infections of domestic animals, experiments have recently been made with rabbits with *Bact. lepisepticum.*

Rabbits developing spontaneous snuffles at the Rockefeller Institute have been examined bacteriologically during life and at autopsy and have been found to carry in their nasal passages *Bact. lepisepticum* as the predominating organism. This microbe has been classed wih the "pasteurella" or hemorrhagic septicemia group and is known as the bacillus of rabbit septicemia. The constant association of the organism with snuffles and its appearance simultaneously with spontaneous snuffles was considered as sufficient evidence to justify the employment of *Bact. lepisepticum* in an attempt to produce snuffles experimentally.

Efforts in this direction were followed by confusing and varied results until young rabbits bred and raised at the Rockefeller Institute from snuffles-free and *Bact. lepisepticum*-free parents were employed. These young rabbits which had not been exposed to snuffles and had always been free from *Bact. lepisepticum* were found regularly to respond to inoculation in a characteristic manner.

Cultures of *Bact. lepisepticum,* grown in broth, were dropped without trauma into the external nares by means of a 2 cc. Luer syringe. Two strains were employed for inoculation.

The less virulent strain was given to one series of five rabbits. Two of these rabbits developed snuffles; the five controls remained normal.

The more virulent strain was given to three series of 6, 12, and 22 rabbits respectively. The following tabulation shows the result:

Number series	Number rabbits injected	Number dying	Number surviving with chronic snuf.	Number surviving. No snuffles
1	6	3	2	1
2	12	3	8	1
3	22	13	4	5

Of the first series of six rabbits, three died, two developed chronic snuffles and survived, and one which had shown no evidence of infection remained well. Of the second series of 12, three died, eight developed chronic snuffles and survived, and one which had shown no evidence of infection remained well. Of the third series of 22, thirteen died, four developed chronic snuffles and survived, and five remained normal. Eight controls remained normal.

In short, 45 carefully controlled young rabbits were inoculated intranasally with two strains of *Bact. lepisepticum.* Nineteen in all died, 16 developed typical chronic snuffles, and 10 resisted infection. The 12 controls, kept under identical conditions with the experimental animals but not inoculated, remained well.

It may be concluded, therefore, that it is possible to produce typical snuffles in rabbits merely by dropping fluid cultures of *Bact. lepisepticum* of sufficient virulence into the nasal passages.

121 (2353)

II. Experimental rabbit pneumonia.

By L. T. WEBSTER (by invitation).

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

It was stated in the previous communication that following intranasal inoculation of *Bact. lepisepticum* into rabbits, a certain number of fatalities ensued. These animals died of pneumonia.

The time of onset of symptoms varied from 24 hours to three days following the inoculation. The rabbits developed signs of respiratory distress, appeared very ill, and showed serous or purulent discharges from the nares. Death ensued in from two to seven days as a rule.

Gross autopsy inspection showed fluid in the pleural cavity varying from a few drops to several cubic centimeters. In the acute cases the fluid was slightly cloudy; in the more prolonged cases it tended to be purulent. The appearance of the pleuræ varied in a corresponding manner from mere dullness to surfaces covered with fibrinous exudate. The lungs usually showed massive areas of consolidation; in early cases, on section, moist and hemorrhagic; in later cases, granular and dry. In some of the older cases the small bronchi exuded pus.

Histologically, the acute cases showed a pleural exudate consisting of fibrin, coagulated serum, and necrotic leucocytes. The pneumonic process seemed to start around the blood vessels where the adjacent alveoli were filled with fibrin, serum, and many red blood corpuscles. Elsewhere little change in the alveoli was seen. The blood vessels were surrounded by sheaths of fibrin containing many polymorphonuclear, mononuclear, and eosinophilic cells. The bronchi were normal. In more advanced cases the alveoli were filled with leucocytes in which the mononuclear type predominated. Finally, the involvement became general; usually an entire lobe was affected. The bronchi contained leucocytes and abscess-like formations occurred.

Bact. lepisepticum was demonstrable in the circulating blood, in the pleural fluid, and lungs.

It has, therefore, been possible to induce experimentally in rabbits the several manifestations of the native respiratory disease called snuffles, pleuropneumonia, and septicemia. Moreover, the experiments show that properly selected rabbits differ in their susceptibility to infection with *Bact. lepisepticum:* a certain number prove refractory, others develop chronic snuffles, but otherwise remain healthy, while still others succumb to an acute fulminating pneumonia or a pneumonia of a slower kind.

122 (2354)

Effect of polar ligations upon the remaining lobe of thyroid gland after the removal of one lobe.

By W. H. BARBER.

[*From the Department of Experimental Surgery, University and Bellevue Hospital Medical College, New York City.*]

A review of the laboratory records of the Class in Surgery, S. 101-102 (1921-1924) shows 63 operations upon the thyroid with data incomplete in 30 and complete in 32. Of the latter, 16 were superior thyroid ligations following hemithyroidectomies, 3 inferior thyroid ligations following hemithyroidectomies, 11 bipolar ligations combined with the removal of one lobe, one bipolar ligation without thyroidectomy and one excision of adventitia from a portion of the carotid artery. It is believed that a superior polar ligation in a hemithyroidectomized dog, in a measure, corresponds with the ligation of the two superior poles in a human hyperplastic goitre. The experimental bipolar ligation may be analogous to the ligation of all four poles in the human gland. The changes in the thyroid remnants under these conditions are thought to be suggestive of the findings in selected human ligations and thyroidectomies.

Of the unipolar ligations less than 20 per cent remained normal while more than 80 per cent revealed hyperplasia of the interstitial epithelial cells or adenomatosis. Of the latter, approximately one-third showed hemorrhage into the thyroid capsule or into the gland or congestion or replacement fibrosis to some degree. After bipolar ligations, the residual thyroid tissue appeared hyperplastic or atrophic in about equal numbers of instances. A few of the latter animals died suddenly within two days after operation.

Parathyroids appeared on section in 21 of the above 32 cases. Of these, approximately one-third remained normal while two-thirds showed congestion or replacement fibrosis. Halsted's observation of degenerative changes in the parathyroids following inferior polar ligations appeared in part borne out in the few instances at hand. In one instance colloid was noted with epithelial cells and acini closely resembling thyroid tissue. In other portions of this same parathyroid gland, the cells occurred in columns and appeared more darkly stained as in normal parathyroids. In most cases, the parathyroids were found either partially or wholly enclosed by the capsule of the thyroid; in exceptional cases a parathyroid or nests of parathyroid cells occurred within the substance of the thyroid gland.

In pregnant or recently pregnant female dogs, the capsular arterial supply was found to be markedly increased and to present a diffuse network of vessels about the whole gland.

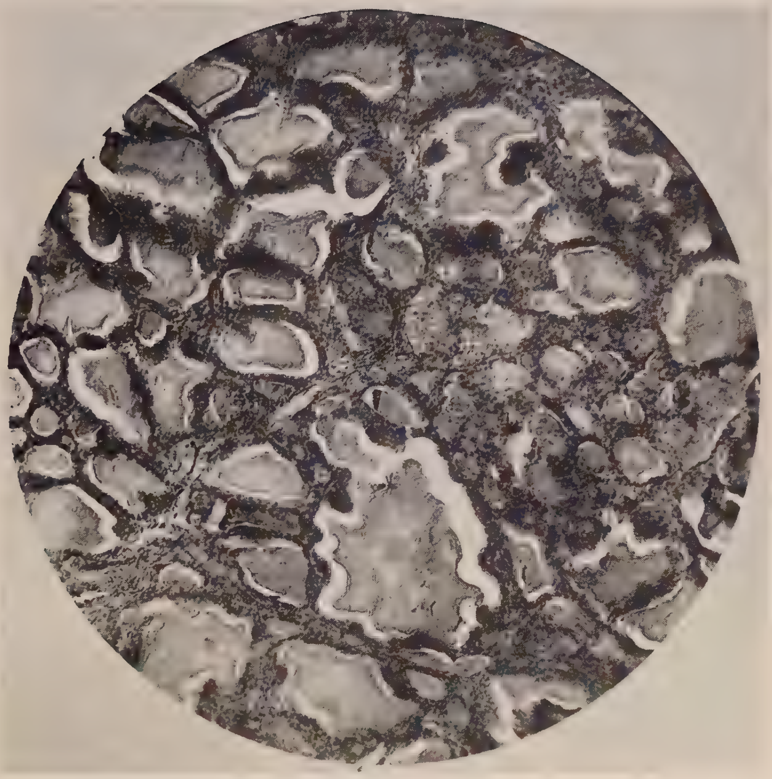

FIGURE 1.

Showing hyperplasia of the acinar cells and papillary ingrowths in a thyroid following hemithyroidectomy and superior pole ligation of the remaining lobe.

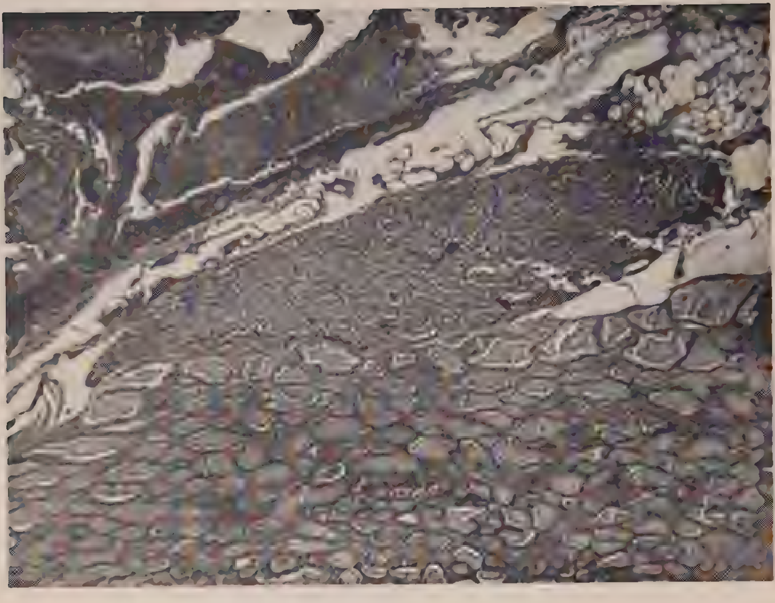

FIGURE 2.

Showing a hyperplastic thyroid and normal parathyroid following ligation of the superior pole of one lobe after removal of the other lobe.

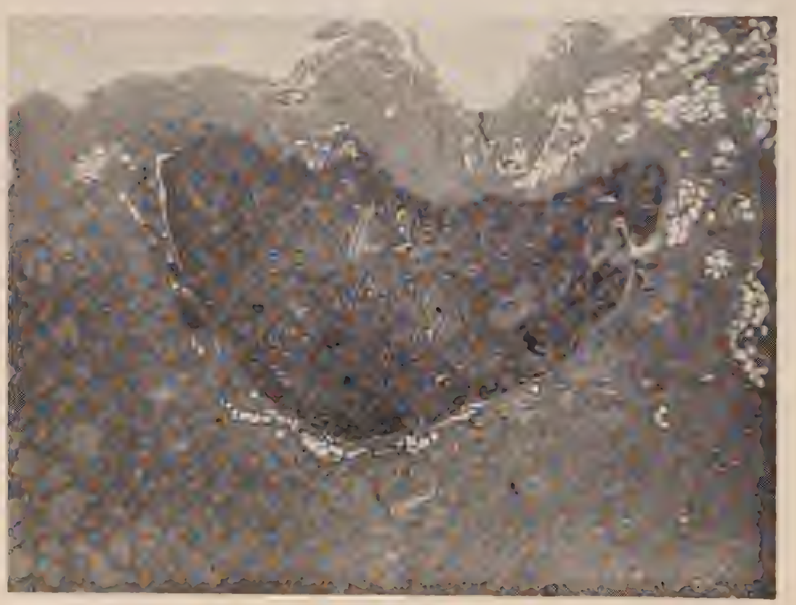

FIGURE 3.

Showing fibrosis about the vessels in the parathyroid and haemorrhage into capsule and thyroid gland after hemithyroidectomy and unipolar ligation.

123 (2355)

A preliminary report on the chemical composition of residue antigen.

By J. HOWARD MUELLER, MARGUERITE WAYMAN
and HANS ZINSSER.

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City, and the Department of Bacteriology and Immunity, Harvard Medical School, Boston, Mass.*]

In a number of preparations of residue antigen obtained from various micro-organisms, chemical analyses have been carried out to find whether or not the presence of proteins, etc., could be definitely excluded. To this end, total nitrogen has been determined, together with amino-nitrogen before and after acid hydrolysis of the preparation.

The bacteria were grown in large agar plates, washed off with saline and treated in the manner previously described in detail,[1] and the resulting material dried in the dessicator.

Specific precipitin tests were given by saline solutions of the dry material with immune rabbit sera, but insufficient quantities were available to attempt to correlate the dilution at which the test became negative, with the chemical composition. For analysis, the entire specimen was carefully weighed, brought into solution in a few cc. of water, and diluted to a definite volume, usually in a 10 cc. volumetric flask, aliquot portions being taken for separate determinations.

Total nitrogen was determined by the micro-kjeldahl method, titrating with 0.02 N solutions, and the amino-nitrogen determinations were carried out by the micro-Van Slyke method.

Phosphorus was determined by Tisdall's[2] method, after wet ashing.

In those preparations in which sufficient material was obtained for duplicate analyses, agreement was fairly uniform. However, we do not believe that absolute accuracy can be claimed for all the determinations, particularly those of amino-nitrogen after hydrolysis, because of the small quantity available for use, and the relatively low content of nitrogen. The values for total nitrogen are probably the most accurate.

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[1] Zinsser and Parker, *J. Exp. Med.*, 1923, xxxvii, 275.

[2] Tisdall, *J. Biol. Chem.*, 1922, l, 329.

It will be observed that there is a rather considerable variation in each type of nitrogen in the different lots. Considering the results as a whole, one sees that the low and variable total nitrogens point to the presence of a nitrogenous substance associated with a non-nitrogenous compound. Concerning the nature of the former, it may perhaps be peptone or polypeptid, since an increase in amino-nitrogen is regularly found after hydrolysis. In connection with the probable non-nitrogenous fraction, of interest is the recent work of Avery and Heidelberger,[3] in which an undoubtedly similar substance prepared by them from pneumococcus broth cultures was shown to be largely a complex carbohydrate. The presence of phosphorus introduces a further possibility of complication, suggesting as it does the presence of nucleic acid derivatives or carbohydrate-phosphate combinations.

The usual color test for proteins and their split-products; *i. e.*, biuret, Millon's, Hopkins-Cole, salicylic acid, ninhydrin and xanthoproteic, were carried out on small quantities of the material in fairly strong solution. Results were uniformly negative, except for an occasional biuret and almost regularly a faint xanthoproteic test.

For the separation and identification of the components of such a mixture, considerable quantities of material will probably be required, quantities practically out of the question if one must depend on micro-organisms of the types used in this study as a source. Since all bacteria which have been investigated thus far, have yielded substances having the same general properties, it is not unreasonable to suppose that the phenomenon may be still more general, and that yeast may produce similar residue antigens, opening the way to a continuation of the work with material which may readily be procured on a large scale. Experiments in this direction have clearly shown that extracts prepared from Fleischmann's yeast will yield a specific precipitate with sera of rabbits immunized to a strain of yeast isolated from this material. A further study of the properties of this extract is now under way.

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[3] Avery and Heidelberger, *J. Exp. Med.*, 1923, xxxviii, 73.

	Wt. of samples	Total nitrogen	Animo nitrogen	Animo nitrogen after hydrolysis	Phosphorus
	mg.	per cent	per cent	per cent	per cent
Tubercle Bacillus	25.32	2.83	.19	1.91	
	22.18	4.50	.31	1.43	
	21.96	1.96	.035	1.53	
Pneumococcus Type I.	18.17	6.23 6.07	0.57 0.53	2.06 2.10	1.24
	14.2	4.45	.34	1.82	—
	28.36	1.45	.16	.47	—
	18.08	3.81	.12	.69	—
	20.8	3.28	.32	1.10	—
	20.7	4.20	—	—	—
Meningococcus	18.86	3.69 3.76	0.41 0.40	3.38 3.49	2.2
	25.1	5.21	—	—	—
	18.06*	2.44	.16	.19	—
	14.88*	2.25	—	—	—
	16.8 *	1.64	.16	.47	—

*Represent successive stages of an attempt to purify a single preparation.

124 (2356)

The preserving effect of alkali on the blood cells of Limulus.

By KENNETH C. BLANCHARD (by invitation).

[*From the Marine Biological Laboratory, Woods Hole, and the Department of Comparative Pathology, Washington University, St. Louis, Mo.*]

1. According to Leo Loeb (1910) alkali may under certain conditions have a preserving effect on the blood cells of *Limulus*.[1] If we add a drop of *Limulus* blood to an excess of an isotonic sodium chloride solution, those amebocytes which come into direct contact with the solution soon send out sharp pseudopodia and lose their granules and become hyaline. Similarly in a watery solution of very weak alkali the cells soon lose their granules and are dissolved; but if both substances are combined, a small

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[1] Loeb, L., *Pflüger's Arch.*, 1910, cxxxi, 465.

amount of alkali being added to the isotonic sodium chloride solution, the cells are preserved much better and their granules may be protected against destruction for a considerable period of time. Alkali may have the same preserving effect even if added to isotonic solutions of anelectrolytes like glucose.

2. This effect of alkali on ameboid cells observed by this method is apparently contrary to the effect of alkali which Loeb and the writer observed by means of the tissue culture method.[2] If the latter procedure is used, alkali causes in the main a more rapid extension and solution of the cells, although even here a preserving effect is noticeable under certain conditions.

Considering this apparent contradiction in the effects of alkali observed, it was necessary to repeat and if possible to extend those earlier experiments, especially in view of the fact that so far they have apparently not been repeated by other investigators.

3. In carrying out such experiments at Woods Hole, last summer, we could confirm the preserving effect of the addition of alkali to isotonic solutions of sodium chloride on the amebocytes of *Limulus.* We found furthermore that alkali has likewise a preserving effect if added to isotonic solutions of glucose, to which a small amount of sodium chloride has been added. Even in combination with an isotonic solution of urea to which a certain amount of isotonic sodium chloride or potassium chloride has been added, alkali may prolong the time during which the cells, and especially the cell granules, are preserved.

4. Addition of alkali does not exert this effect through a direct osmotic action; the amounts of alkali which are thus effective are too small for this purpose, inasmuch as the preserving action is obtained in a solution of 0.01 to 0.005 N NaOH. As to the mode of action through which alkali exerts this influence, Loeb suggested that it might be due to an inhibiting effect of alkali on certain cell activities, which somehow may favor the solution of the granules. We noticed that alkali under the conditions of this experiment diminishes or prevents the ameboid activity of the cells, and also the extension of the blood cells which usually occurs when in contact with the glass; and it is especially the latter process which favors the solution of the granules. Alkali seems not only to restrict or inhibit the

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[2] Loeb, L., and Blanchard, K. C., *Am. J. Physiol.*, 1922, lx, 277.

formation of the typical tongue pseudopodia, but also that of the balloon pseudopodia and the circus movements which were produced in mixtures of urea and potassium chloride.

The fact that other agencies which inhibit these processes (KCl, NH_4Cl for instance) likewise exert a certain preserving effect on the blood cells seems to support this interpretation. However, further experiments must decide whether in addition other factors may not contribute to this preserving effect of alkali.

125 **(2357)**

The effect of acid on the amebocyte tissue of Limulus in tissue cultures.

By LEO LOEB, JESSIE M. BIERMAN and ELIZABETH GILMAN.

[*From the Department of Comparative Pathology, Washington University, St. Louis, Mo., and the Marine Biological Laboratory, Woods Hole, Mass.*]

In previous publications[1] we have shown, (1) that a small amount of acid added to an isotonic sodium chloride solution exerts a certain preserving effect on the amebocytes in a drop of *Limulus* blood surrounded by an excess of the solution. (2) That in tissue cultures of amebocyte tissue there occurs not only an active migration of the amebocytes into a sodium chloride solution, which has been made slightly acid, but that under certain conditions the addition of acid may increase the duration and intensity of this migration, which is followed by, or associated with, a formation of tissue-like structures not unlike those produced by the mesenchyme of vertebrates.[2] (3) That this effect of acid is due to its action on the cells, which consists in an increase in contraction and consistency of the protoplasm. (4) Inasmuch as we had previously shown that there exists a parallelism between the effect of various agencies on the state of contraction and consistency of the amebocytes on the one hand, and on the other hand, on the character of ameboid movement, the shape of pseudopods, the mode of movement of the granuloplasm, the power of resistance to the stereotropic tendency on the part of these cells, we found in accordance with these observations under the influence of acid a sharpening and lengthening of the pseudopods and a tightening of the granuloplasm and a greater state of contraction of the cells. (5) We found that it is possible, through grading the amount of acid added, to grade correspondingly the effects of the acid on the consistency of the cells in such a way that all gradations could be obtained from the extreme condition of round contracted cells, too hard to carry out ameboid movements, and contracted cells sending out very long, often multiple thread pseudopodia, to a medium state in which the plasticity of the cell is greater and the ordinary tongue pseudopods are formed, and finally to the other extreme condition of cells which are very soft, which spread out readily and soon degenerate (Loeb & Blanchard).

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[1] Loeb, L., *Folia Haematologica*, 1907, iv, 313. *Pflüger's Arch.*, 1910, cxxxi, 465. *Washington Univ. Studies*, 1920, v, 8. *Scient. Series* 3. *Am. J. Physiol.*, 1921, lvi, 140. *Science*, 1922, lvi, 237. Loeb, L., and Blanchard, K. C., *Am. J. Physiol.*, 1922, lx, 277.

[2] We use the term "outgrowth" to designate these effects.

In continuing these investigations during the summer of 1922 and 1923, at Woods Hole, we found (1) that, if we add small amounts of hydrochloric acid to solutions of NaCl, a steady decrease in the hydrogen ion concentration takes place in the fluid surrounding the tissue. This diminution in the hydrogen ion concentration begins shortly after adding the tissue, increases in the next few hours and continues for from one to two days or longer. The intensity of the loss varies in different cases in accordance with the character of the tissue, with the size of the piece of tissue used, and probably also with the amount of outgrowth that has taken place. (2) Outgrowth could be obtained when the pieces of tissue were placed in solutions of HCl varying in strength from M/1000 to M/8000 HCl. The optimal concentration varied in different cases according to the variable factors stated above.[3]

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[3] The method of colorimetric determination of hydrogen ion determinations used by us is similar to the one described by Felton, L. D., *J. Biol. Chem.*, 1921, xlvi, 299. However, in this paper, of which we learned only recently, several improvements are suggested which we intend to use next summer. Since our first observations on the effect of acid on the movements of cells in tissue culture were published (1920), the following papers which are of interest in this connection appeared: Chambers, R., PROC. EXP. BIOL. AND MED., 1921, xix, 37; Lewis, M. R., and Felton, L. D., *Science*, 1921, liv, 636; Lewis, M. R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 373; Fenn, W. O., *J. Gen. Physiol.*, 1922, v, 169; Loeb, J., *J. Gen. Physiol.*, 1922, v, 231.

(3) The beneficial effect of acid on the outgrowth of amebocyte tissue may be observed in hypertonic as well as in hypotonic solutions of sodium chloride. In certain respects the effects of acid and of hypertonicity are additive, while those of acid and hypotonicity are antagonistic. However, acid merely diminishes to some extent the injurious effects of hypotonicity without being able to prevent them. A concentration of acid which still permits outgrowth in hypotonic solutions may be too strong for this purpose, if added to hypertonic solutions.

(4) We have found previously that while fresh *Limulus* serum is usually a favorable medium for the movement of amebocyte tissue, *Limulus* serum, from which the proteins have been removed through heat, has lost to a great extent its favorable character. This loss can partly be made good if we add acid to the protein-free filtrate of *Limulus* serum. The filtrate then regains some of the effects of fresh serum; however, it is necessary to add much larger quantities of acid to filtrate than to sodium chloride solutions on account of the buffer substances present in this filtrate of *Limulus* serum.

(5) Acid acts primarily on the amebocyte tissue by increasing the consistency and state of contraction of the cells. This effect has two consequences: (1) the cells are made less plastic and their ameboid activity is thereby decreased; (2) the cells are to a certain extent protected against injurious effects of the surrounding fluid and particularly the secondary injurious effects of the stereotropic reaction are delayed.

The first one of these two effects tends to make the outgrowth in acid solutions smaller than in corresponding neutral solutions, while the second one tends to preserve the cells for some time and thus to enable the tissue in certain cases to be still active at a time when in the neutral control solutions degenerative changes have cut short further active outgrowth of the tissue. Thus the outgrowth in an acid medium can under certain conditions surpass that of the control tissue in a neutral medium.

The maximum outgrowth is usually obtained when the tissue is sufficiently soft to allow a limited number of cells to spread out and hyalinize, and when at the same time a preserving effect of the acid is still noticeable. With the gradual decrease in the acidity of the medium and with the tendency of tissue even in neutral solution to become soft and to spread out in the course of time, the optimum condition for outgrowth is obtained in later periods of the experiment, in preparations in which the concentration of acid was at first too great to allow an active migration of the cells; at a still later period the optimum of consistency is passed in this solution, the cells become still softer and degenerative processes predominate. Thus the optimum passes in the course of time from preparations in which the concentration of acid was relatively low to preparations which had originally a higher concentration of acid.

Whether in a specific case the outgrowth in acid surpasses in duration and ultimately also in intensity that in the control solutions, depends upon a number of variable factors, especially important among which is the original power of resistance of the tissue to injurious factors.

126 (2358)

Presence of toxic and insulin-like substances in oranges, grape fruit and lemons.

By N. F. FISHER and EARL B. McKINLEY (by invitation).

[*From the Departments of Physiology and Bacteriology, Baylor University College of Medicine, Dallas, Texas.*]

During the past few months Winter and Smith,[1] Best and Scott,[2] Funk and Corbitt,[3] Collip,[4] Perry,[5] and Dubin and Corbitt[6] have reported the presence of a substance in yeast and in plants and vegetables, which causes a fall in blood sugar upon injection into normal rabbits. In view of these results the possible presence of this substance in various fruits suggested itself to us.

Various methods of extraction were used with oranges, grape fruit and lemons with the following results:

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[1] Winter, L. B., and Smith, W. J., *J. Physiol.*, 1922, lvii, 100.

[2] Best, C. H., and Scott, D. A., *J. Metabol. Research*, 1923, iii, 177.

[3] Funk, C., and Corbitt, H. B., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 422.

[4] Collip, J. B., *J. Biol. Chem.*, 1923, lvi, 513; 1923, lvii, 65.

[5] Thalhimer, W., and Perry, M. C., *J. Am. Med. Assoc.*, 1923, lxxx, 1614.

[6] Dubin, H. E., and Corbitt, H. B., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 16.

1. In every case a blood sugar increasing and a blood sugar decreasing principle was clearly demonstrated.
2. The type of curve obtained was dependent upon the varying amounts of either substance present.
3. Predominance of the toxic fraction (blood sugar increasing substance) invariably produced a preliminary rise in the blood sugar which was followed by a fall below normal. The period of delay in the fall of blood sugar varied with the amount of the toxic substance present.
4. By methods of purification, which will be reported later, it is possible to separate to a very large degree the hypoglycemia-producing principle from the hyperglycemia-producing substance; at least by these methods the double effect described when both substances are present, can be eliminated and a continuous rise or fall of the blood sugar can be obtained.
5. The hypoglycemia-producing principle and the hyperglycemic substance are present in the juice, pulp and rind of the fruits we have examined.
6. The hypoglycemia-producing principle acts similar to insulin. No delayed action has been observed as described for "Glucokinin".

The above findings appear to be in agreement with those reported by Dubin and Corbitt for vegetable extracts. At present it is impossible to state whether the slightly delayed fall in blood sugar of normal rabbits is a qualitative or a quantitative phenomenon of the toxic fraction.

127 (2359)

The nature of human isohemagglutinogens.

By L. G. HADJOPOULOS and REGINALD BURBANK (by invitation).

[*From the Department of Laboratories, Beth Israel Hospital, New York City.*]

In a previous communication on the nature of specific hemolysins, one of us[1] has dealt on the complexity of erythrocytes as antigen and endeavored to demonstrate that the cell stroma is the main, if not the sole source of hemolysinogens. In this paper, as a supplementary study, we have undertaken to demonstrate that isohemagglutinogens, in a similar way, originate in the cell stromata.

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[1] Hadjopoulos, L. G., *Arch. Int. Med.*, 1921, xxvii, 441.

To obtain cell stromata in a more or less pure condition and individually from each of the four different types of bloods we used the same technique as discussed in our previous communication. In short it consists of: *First,* isolating the four types of erythrocytes. Samples of blood, left over from Wassermann reactions were used for this purpose. *Second,* filtering through gauze and centrifugalizing the saline suspension of broken clots. This step was repeated until the supernatant fluid was free of serum ingredients. *Third,* hemolyzing the final sediment in 0.45 per cent saline solution and sedimenting stromata by centrifugalization. To get rid of hemoglobin further sedimentations were done in physiological saline to avoid deterioration of cell stromata.

The final product, when examined under the microscope, should show the cell stromata intact, more or less globular, varying in size from that of a blood platelet to half the size of an erythrocyte and devoid of hemoglobin. Presence of hemoglobin means deficient "dehemoglobinization", which will necessitate repetition of step three until dehemoglobinized cells (cell stromata) look almost transparent.

TECHNIQUE

The four types of serums are isolated, washed free of their cellular constituents and divided in amounts of 0.20 cc. into four small tubes. This gives sixteen tubes, arranged in four rows and of four tubes each. The first row of tubes, representing the four types of serums, receives a sufficient amount of type I cell stromata, the second row, type II cell stromata, etc. The cross absorption test is then carried out in the incubator for 30 minutes and over night in ice box. The following morning all tubes are centrifugalized and the clear serums transferred in the same order to another set of sixteen tubes. The first row now represents type I absorbed serum, the second type II, etc.

A preliminary test of the degree of absorption is done by taking a drop of serum from tube No. 3 second row, a drop from tube No. 2 third row and one from tube No. 1 fourth row. After placing these drops in their order on a clean slide a drop of group III cell suspension is mixed with drop No. 1, group II cells with drop No. 2 and group I cells with drop No. 3. The slide is now kept at room temperature for 30 minutes under a petri dish cover to prevent drying. If no agglutination occurs at the end of this time it is safe to consider the absorption as complete.

The original sixteen tubes are now centrifugalized at high speed for ten minutes and then arranged in their original order. Eight clean and dry glass slides are set in succeeding order with a cover for each. The first slide gets four drops from tube No. 1 and under these another four drops from tube No. 2. In a similar way the other seven slides are prepared and covered. Of the four rows of drops, with sixteen in each row, No. 1 gets a drop of type I cell suspension, No. 2 a drop of type II cells, etc. At the end of 30 to 40 minutes the results are read macroscopically and, if need be, also microscopically. Table I demonstrates our results.

TABLE I.

Results of cross absorption tests with cell stromata.

Type serums absorbed with type stromata	Unabsorbed agglutinin after absorption	Washed, intact erythrocytes							
		i	(AB)	ii	(A)	iii	(B)	iv	(0)
		Theor.	Act.	Theor.	Act.	Theor.	Act.	Theor.	Act.
I - i	0 - AB=0	—	—	—	—	—	—	—	—
I - ii	0 - A =0	—	—	—	—	—	—	—	—
I - iii	0 - B =0	—	—	—	—	—	—	—	—
I - iv	0 - 0 =0	—	—	—	—	—	—	—	—
II - i	b - AB=0	—	—	—	—	—	—	—	—
II - ii	b - A =b	+	+	—	—	+	+	—	—
II - iii	b - B =0	—	—	—	—	—	—	—	—
II - iv	b - 0 =b	+	+	—	—	+	+	—	—
III - i	a - AB=0	—	—	—	—	—	—	—	—
III - ii	a - A =0	—	—	—	—	—	—	—	—
III - iii	a - B =a	+	+	+	+	—	—	—	—
III - iv	a - 0 =a	+	+	+	+	—	—	—	—
IV - i	ab - AB=0	—	—	—	—	—	—	—	—
IV - ii	ab - A =b	+	+	—	—	+	+	—	—
IV - iii	ab - B =a	+	+	+	+	—	—	—	—
IV - iv	ab - 0 =ab	+	+	+	+	+	+	—	—

Note. Under the first column the capital Latin numerals stand for the various types of serums; small numerals for the various group stromata used to absorb the corresponding serums. In the second column, the small letters stand for the agglutinins and the capitals for the agglutinogens. In the other four columns the small Latin numerals represent the type of the intact erythrocytes. The sign of plus means agglutination; the negative sign absence of agglutination. Under each column there are two readings: the first is what we should get theoretically and the second what we actually got in the course of our experiments.

The close parallelism of the theoretical and actual findings leaves no doubt in our opinion that "dehemoglobinized" cells (cell stromata) are the main, if not the only source of hemagglutinogens.

128 (2360)

Serological examination of a species-hybrid.

By K. LANDSTEINER and J. VAN DER SCHEER.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

Although of some general interest, the question of the heredity of species-specific properties in animals as indicated by serum reactions has not yet been investigated. While some difficulty exists in discriminating substances derived from closely related species by precipitins, it is easy to do so by the use of hemagglutinins.

After producing agglutinins in rabbits with the blood, for instance of the horse or donkey, one obtains, by absorption with the heterologous blood, solutions which act specifically on the blood of the species used for immunization. In this way the blood corpuscles of a species-hybrid, namely the mule, were examined by means of immune sera against horse, donkey, and mule blood. Of the several possibilities, such as conformity of the blood of the hybrid with that of one parent, a combination of the properties of both, or formation of new substances, the one which actually occurs is the inheritance of agglutinable substances of each parent.

It remains to be estabilished whether the results are different in the cross between the mare and the jack and the reciprocal cross.

We hope to extend the studies to cases of fertile hybrids.

129 (2361)

Observations on the action of novasural in heart disease.

By J. HAMILTON CRAWFORD and J. F. McINTOSH (by invitation).

[*From the Hospital of the Rockefeller Institute for Medical Research, New York, City.*]

Novasural is a preparation containing 33.9 per cent of mercury bound in a large molecule. It is given as a 10 per cent solution in doses of 1 to 2 cc. It was originally introduced as an antisyphilitic, but its marked action on urine output suggested its use as a diuretic. It was first used for this purpose by Zeiler.[1] Later studies have been published by Saxl and Herlig,[2] Mühring[3] and Nonnenbruch.[4] These writers agree as to its efficiency as a diuretic in selected cases, but fail to agree on the mechanism of its action.

In our cases novasural has been administered to patients with severe heart failure and edema in whom digitalis had failed to relieve the edema. It has been administered in 1 to 2 cc. doses intramuscularly and has been followed by a striking diuresis—the output often rising from 300 cc. to 2000 to 3000 cc. per diem.

The diuresis as a rule starts within two hours and attains its maximum in three to nine hours. The more serious the case, the more delayed is the time of the maximum diuretic effect. The action passes off in about 24 hours.

Studies have been made of the urine, blood, and when possible the edema fluid before, during and after the diuresis. During the diuresis the total output of chlorides in the urine is increased and the concentration is also increased. The total urea and ammonia in the urine may be increased, but the concentration is reduced. The blood chlorides are slightly reduced and the chlorides in the edema fluid follow closely those of the blood. The blood urea shows practically no alteration.

There has been marked clinical improvement in all cases and no untoward effects have been observed.

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[1] Zeiler, *Münch. med. Wchnschr.*, 1917, xxxix, 1257.

[2] Saxl and Herlig, *Wien. klin. Wchnschr.*, 1920, xxxiii, 943.

[3] Mühring, *Münch. med. Wchnschr.*, 1921, lxviii, 1447.

[4] Nonnenbruch, *Münch. med. Wchnschr.*, 1921, lxviii, 1282.

The urine was examined daily for albumen, red blood cells and casts to study whether any damage was done to the kidneys. No evidence of any such damage was found during the month following the injections. In fact these findings improved along with an apparent improvement in the circulation.

130 (2362)

Effect of menotoxin on the viability of spermatozoa.

By DAVID I. MACHT and C. F. ELVERS.

[*From the Pharmacological Laboratory and the Brady Urological Clinic, Johns Hopkins University, Baltimore, Md.*]

Macht and Lubin have called attention first in these Proceedings and again in a more extensive publication appearing elsewhere to the remarkable phytopharmacological properties of menotoxin, the toxin present in the blood and other secretions of women at the beginning of menstruation. It was pointed out that while this toxin was extremely poisonous for plant protoplasm it was comparatively very little poisonous for animal tissues, although differences in toxicity between normal and menstrual blood for animals have been found. This difference between the phytopharmacological and zoöpharmacological actions of menotoxin were shown to hold for microscopic organisms as well. Thus menstrual serum was quite toxic for bacteria such as *B. coli* which are generally classed with plants on the one hand, and the same serum was but slightly toxic for trypanosomes which are more closely related to animals, such as the flagellates, on the other. In this connection it was interesting to inquire into the toxicity of menotoxin for spermatozoa.

Fresh and actively motile spermatozoa were obtained from men and the effect of various toxins was studied on the same in hanging drop preparations. It was found that menstrual serum was not more toxic for spermatazoa than normal serum. The authors made further studies on the effect of various chemicals which seem to be closely related to menotoxin. As has been pointed out by Macht and Lubin, menotoxin exhibits chemical and pharmacological properties resembling oxycholesterol on the one hand, and the bile acids on the other hand. Experiments were made with emulsions of oxycholesterol in normal serum, with solutions of cholic acid and other closely related chemicals. It was found that neither oxycholesterol nor cholic acids had any marked toxic effect on spermatazoa. The results obtained with these various substances agreed with the findings in the case of menstrual serum and further substantiate the close relationship between the chemical nature of menotoxin on the one hand and oxycholesterol and cholic acid on the other hand.

131 (2363)

The effect of corpus luteum on behavior of rats.

By DAVID I. MACHT and DOROTHY W. SEAGO.

[*From the Pharmacological and Psychological Laboratories, Johns Hopkins University, Baltimore, Md.*]

The effect of corpus luteum extracts were studied on the behavior of rats in the circular maze according to the methods described by the senior author elsewhere. Sixteen young adult, female rats were trained in the maze until they could solve the problem in the shortest period of time without committing any errors. They were then given injections subcutaneously or intramuscularly of "lutein", a protein-free extract of corpus luteum of the sow, prepared by Hynson, Westcott and Dunning. It was found that injections of doses varying from 0.2 to 0.5 cc. of lutein in the rats the average weight of which was about 150 gm. produce a distinct stimulation in their muscular activity and cerebro-spinal efficiency. This effect was noticeable 15 minutes after injection of the extracts and persisted for 24 hours. Injections of the corpus luteum in ovariectomized rats produced a similar stimulation in the behavior of the animals. A few experiments performed with injections of extracts of whole ovary yielded the same results. Further details to appear elsewhere. (Journal of Comparative Psychology).

132 (2364)

Effect of labyrinthine stimulation on the vagus control of the heart.

By RALPH ALMOUR and MORRIS H. KAHN.

[*From the Oto-laryngological and Cardiovascular Departments, Beth Israel Hospital, New York City.*]

The work presented in this paper is the first of a series of studies which were undertaken with the view of determining the existence of a reflex path between the vestibular nerve and the autonomic or visceral nervous system. This investigation was prompted by a two-fold purpose: to establish, if possible, the existence of a tonic influence of the kineto-static labyrinth on the visceral nervous system, and to determine the physiological relationship between the two; and to establish the presence of a phenomenon which would be applicable to practical use from a clinical standpoint.

The labyrinth exerts a tonic influence on the general body musculature through its intercommunications with the somatic nervous system. The effects of the labyrinth on the visceral nervous system are not nearly as obvious nor as easily demonstrable as its effects on the somatic nervous system. For example, the stimulation of the vomiting center which occurs during the performance of the labyrinthine tests, or during an attack of seasickness, may be the result of cerebral impulses produced by the cumulative nausea, or as some authorities believe, may be produced by a direct reflex action of the hyperactivated labyrinth upon the center in the dorsal nucleus of the vagus.

That an interrelation exists between the visceral nervous system and the labyrinth is apparent when one considers that tinnitus is a common symptom accompanying disturbances of the viscera in other parts of the body. Woakes[1] considers that impulses from the stomach pass by way of the vagus nerve to the inferior cervical ganglion, where a communication is established with the nerves which regulate the amount of blood supply to the inner ear through the internal auditory artery. The vascular changes thus produced, cause the tinnitus. This, of course, is only a reflex involving the sensory portion of the vagus. That a like reflex might possibly exist wherein the labyrinth and the vestibular nerve compromise the receptor and afferent arc, and the visceral nervous system and the efferent arc, has led us to investigate the effects of labyrinthine stimulation on the various components of the autonomic nervous system.

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[1] Woakes, *Deafness, Giddiness and Noises*, p. 135; quoted by Kerrison, *Diseases of the Ear*, Lippincott, 1913.

EFFECT OF STIMULATION OF THE LABYRINTH ON THE HEART.

The ramifications of the vagus nerve and its association with the various nerve tracts are so complex that they embrace practically all the vital functions. The oculo-cardiac reflex evidences such a pronounced physiological relationship between the eye and the heart through the vagus, that it might theoretically be conceived that the other special senses have a corresponding intimacy with it.

In a study of the psycho-galvanic reflex,[2] it has been shown that stimulation of the special senses will register a reaction in the pulse and respiration. This has been done by means of the string galvonometer. The cause of the deflections produced in the string of the galvonometer was an external stimulus resulting in a muscular effect.

By this method, Albrecht[3] was able to distinguish organic from inorganic disturbances in hearing. He allowed a current of a given strength to pass through the subject, and connected him to a coil galvanometer. Then, upon applying sound stimuli, there were observed vibrations of the string which depended upon endosomatic processes. These vibrations can be distinguished from those produced by other causes (for instance, muscular contractions) by the latent period of one or two seconds which intervenes between the time of stimulation and the appearance of the vibrations.

Van Iterson[4] suggested the importance of these examinations in the testing of the auditory apparatus. He showed that by applying sound stimuli, in a person with a functionating auditory apparatus, a deflection of the galvanometer could be observed every time a sound was heard. This appeared after a short latent period. In a deaf person, this psychic effect was not produced. The reaction, in each case examined, occurred absolutely independently of the patient's will.

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[2] Wiersma, E. D., *Verslagen Koninklijka Academie van Wetenschappen*, 1915.

[3] Albrecht, D. W., *Arch. f. Ohren, Nasen u. Kehlekopfheilkunde*, 1917, ci. 1.

[4] Van Iterson, C. J., *Acta Oto-laryngologica*, 1920-21, ii, 174.

We have endeavored, by means of the electrocardiogram, to observe what effect the stimulation of the kineto-static labyrinth produced upon the vagus control of the heart. The method of procedure was as follows: The patient was attached in the electrocardiographic circuit, and a normal record in all three leads was taken after the resistance of the body in the circuit had been estimated. The labyrinth was stimulated by the caloric method, using water at 68° C.

After the normal records were taken, irrigation of the ear, either right or left as indicated in the chart, was then begun simultaneously with the taking of an electrocardiographic record in Lead II. In the first several patients, the record was continued throughout the entire test. We later found it equally satisfactory to take a short strip at the beginning of the douching, another as soon as nystagmus was noted, a third strip at the first evidence of vertigo, and a fourth at the onset of nausea. Although careful notation was made at the time when these phenomena appeared clinically, the electrocardiogram in most of the cases, showed more exactly the time of occurrence.

In analyzing the tracings, it is necessarily desirable to establish and briefly review what may be the possible effects of vagus stimulation upon the electrocardiographic record. One of the most conspicuous effects of vagus stimulation is upon the sinus node, causing a slowing of the heart rate. An equally common effect is an increase in the conduction time, *i. e.,* a relative blocking between the P (auricular) and R (ventricular) waves. The occurrence of nodal rhythm is perhaps next frequent in occurrence. The other forms of irregularities and abnormalities in the heart mechanism caused by vagus stimulation are not so common, and need not be considered in this study.

Analyzing the electrocardiographic films, we find no evidence of any changes in the character or rate of the heart. No cases, wherein the ears were either normal or abnormal, evinced any changes that could be attributed to vagus action, even where there was reason to believe that vagotonia existed. Two of the cases were asthmatics with signs of a vagotonic condition. One of the cases tested was a patient in whom one labyrinth was shown to be non-functioning, both in its cochlear and kineto-static portions. Records were taken of the effect of the caloric test on the electrocardiographic picture, each ear being subjected to the douching while the patient was in the electric circuit. The same findings occurred for the testing of both the active and destroyed labyrinth.

We therefore conclude from our experiments, that no reflex nerve path exists from the ear to the heart by way of the vagus nerve. That portion of the dorsal nucleus of the vagus which ccmprises the cardiac center of the vagus is not influenced by stimulation of the labyrinth by the caloric method, either during the period of reflex activity (nystagmus) or after consciousness reacts to the stimulation in the form of vertigo and nausea.

ABSTRACTS OF COMMUNICATIONS.

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Fifth Meeting.

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133 (2365)

The viability of hemolytic streptococcus in certain solutions containing gelatin.

By FRANK L. MELENEY and ZUNG-DAU ZAU.

[*From the Department of Surgery, Peking Union Medical College, Peking, China.*]

For certain quantitative biologic tests it is necessary to use a suspending medium in which the streptococcus will survive without increase or diminution in numbers for a considerable length of time.

Falk has recently reviewed the extensive literature on the subject of the inhibitory effect of the cations of various salts on the biologic activities of bacteria and other cells. He concludes that balanced solutions containing two or more salts in the proper proportions preserve the life of bacteria better than either distilled water or solutions containing single salts unless they are very dilute.

Robertson, Sia and Woo have recently found that 0.1 to 0.125 per cent gelatin in distilled water or Locke's solution greatly increases the length of life of the pneumococcus in these fluids.

With these facts as a basis, experiments with a hemolytic streptococcus obtained from an acute gangrenous infection of the thigh have been carried out with six different fluids: 1, distilled water; 2, "normal" saline; 3, Locke's solution; 4, a balanced solution containing 1 per cent sodium chloride and 0.05 per cent calcium chloride ("20-1"); 5, 0.2 per cent sodium citrate; 6, a balanced solution containing 0.5 per cent sodium chloride, 0.5 per cent potassium chloride, 0.5 per cent calcium chloride and 0.1 per cent magnesium chloride (Zeug's mixture).

Each fluid was prepared both with and without 0.1 per cent gelatin and titrated after autoclaving to pH 7.8. The streptococcus was grown in 1 per cent dextrose digest broth, washed twice and then suspended in the solution to be tested at a standard concentration, approximately one billion organisms per cubic centimeter. This was diluted down seven times with the fluid to be tested, each dilution containing a tenth of the number in the previous dilution so that the final dilution was estimated to contain 100 organisms. Each dilution was then divided up into one cubic centimeter quantities and allowed to stand, one series at room temperature (19-22°) and another series at incubator temperature (37.5°). Immediately after the dilution process was finished and at intervals of 6, 12, 18, 24, 48, and 72 hours, 5 cubic centimeters of digest broth were added to a representative tube of each bacterial dilution, both in the room temperature series and in the incubator series. These tubes were then incubated and growth or death was noted. Negative results were not recorded until after 72 hours incubation. In every case, irrespective of the fluid used, those containing gelatin maintained life longer than the corresponding fluid without gelatin. At room temperature in the gelatin Locke's, gelatin citrate and the gelatin "20-1" solutions the streptococci survived for 72 hours at dilutions with an estimated concentration of 100 cocci per cubic centimeter. At incubator temperature these solutions maintained life in the highest dilutions for twelve hours. These solutions may, therefore, be used for certain biologic tests. The other three gelatin solutions, namely Zeug's mixture, water and saline, were not as efficient and in the solutions without gelatin there was very rapid death especially at incubator temperature.

When the six gelatin solutions were tested against each other it was found that the gelatin citrate solution maintained life in the two highest dilutions at room temperature until the end of the test—15 days. The others in the order of efficiency were Locke's, "20-1", Zeug's mixture, distilled water and "normal" saline. In the "normal" saline with gelatin the streptococci died after four days in the sixth dilution and after two days in the seventh dilution. Thus, although gelatin protects the streptococci in these solutions the protection is not absolute. After a time death takes place in about the same relative order as in the solutions without gelatin according to the relative toxic action of the fluids. The sodium cation in the citrate solution is not sufficiently concentrated to be toxic or its toxic effect is inhibited by the citrate anion, which may be nutrient.

By testing gelatin water and gelatin Locke's solution at different levels of hydrogen ion concentration it was found that the streptococci will survive in these solutions in the highest dilution, for at least three days in a zone ranging from pH 7.4 to pH 8.2 or even wider. If the solutions are adjusted before use to pH 7.8 they will maintain their reaction within that zone.

In experiments carried out in an effort to explain the protective action of gelatin there was found to be a protection against the mechanical injury of the diluting process. But the protection is more than that, for death takes place much more rapidly at incubator temperature than at room temperature, after the same degree of mechanical injury. There may be a coating of the organisms delaying the entrance of toxic substances. Probably also the gelatin has a slight nutrient action, enough to permit a slow metabolism or prevent autolysis, but not enough to cause a multiplication of the organisms. In the 1 per cent gelatin Locke's and gelatin citrate solutions the streptococci maintain an even numerical level for from 15 to 24 hours at room temperature and then gradually die off. At concentrations of 0.5 per cent and 1.0 per cent, however, they actually multiply.

134 (2366)

The action of morphine, heroin, and codeine on respiration.

By CARL F. SCHMIDT.

[*From the Laboratory of Pharmacology of Peking Union Medical College, Peking, China.*]

In a previous series of experiments[1] on cats, it was found that the action of morphine and heroin on respiration was practically limited to a selective depression of the expiratory mechanism, and that codeine produced only stimulation of the spinal cord. These experiments were repeated upon decerebrated and anesthetized dogs, and upon anesthetized rabbits, inducing active expiratory efforts by inhalations of a constant CO_2-air mixture, and recording them by means of a record of intrathoracic pressure, using an esophageal balloon or a pleural cannula; blood pressure and respiratory volume were also recorded.

The results confirm those obtained with cats, namely, morphine and heroin have a selective action on the expiratory mechanism of dogs and rabbits as well as cats, while no constant depressant action could be detected with codeine.

With morphine, dogs showed passive expiration after 2 to 6 mg. per kilo, as a rule, and larger doses had no further effect until circulation was depressed; occasionally very large doses—300 mg. per kilo—reversed this effect and produced increased reflex excitability, but convulsions did not occur, even in decerebrated animelas. Depth of respiration was often increased as the rate was slowed. With rabbits, expiratory depression began with 1 mg. of morphine and was usually complete after 3 to 5 mg., but depth was more frequently decreased than was the case with dogs. Larger doses sometimes reversed this effect, and caused increased reflex excitability, with a return of active expiration, but the circulation of the rabbit was not depressed as much by morphine as was that of the dog or cat.

With heroin, 0.05 mg. per kilo sometimes made expiration completely passive in the dog, and depth of breathing was usually increased as the rate was slowed. Larger doses depressed circulation but had no further direct effect on respiration. In one experiment heroin depressed circulation but not respiration, and tetanic convulsions followed 80 mg. per kilo. In rabbits, expiratory depression and slowing of the rate of breathing began with 0.125 mg., and 0.25 to 0.5 mg. usually produced maximal slowing, though expiration was somewhat active when the rate was slowed, and depth was often decreased, but the slowing could always be completely removed by emptying the lungs more completely, as by pressure on the chest, suction on the tracheal cannula, or a partial pneumothorax. Apparently the expiratory efforts were too weak to arouse excitatory reflexes in the lungs, or the inspiratory cells were also depressed. Heroin also had less depressant action on the circulation of the rabbit than on that of the dog or cat.

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[1] Schmidt and Harer, *J. Exp. Med.*, 1923, xxxvii, 47 and 69.

Codeine, in dosage from 0.1 mg. to over 100 mg. per kilo, failed to slow respiration or make expiration passive in dogs or rabbits, and tetanic convulsions were the only definite result. In one recent experiment on a decerebrated cat, 5 mg. of codeine produced slowing and expiratory depression, but 25 mg. were required to remove the expiratory response to CO_2, while convulsions followed 35 mg.

It appears, therefore, that a selective depression of expiration is the characteristic action of morphine and heroin on the respiration of dogs and rabbits as well as of cats, though this action is not as clean-cut in the rabbit as in the other animals. That this effect may be responsible for the selective action of these drugs on the rate of breathing is shown by the acceleration which follows more complete emptying of the lungs, and this has been consistently observed in dogs and rabbits, as well as in cats, when the rate was slowed by morphine or heroin. It is possible that a similar selective action on expiration may explain the specific action of these drugs in cough and dyspnea in man. Codeine may possess a similar action on expiration, but it is certainly weaker and is usually masked by the action of the drug on the spinal cord.

135 (2367)

The action of respiratory stimulants in morphine and heroin poisoning.

By CARL F. SCHMIDT.

[*From the Laboratory of Pharmacology of Peking Union Medical College, Peking, China.*]

In the experiments on cats previously reported, it was apparent that the action of morphine and heroin on respiration was limited to a depression of expiration, and that an extremely slow rate and respiratory failure depended on a primary depression of the circulation. These observations have been confirmed in every case in experiments on dogs, and in most experiments on rabbits, though in one experiment heroin caused temporary respiratory failure and asphyxial effects on the blood pressure of a rabbit, and circulatory depression was usually less marked in rabbits after morphine or heroin than in dogs or cats. The extremely slow regular breathing of advanced morphine or heroin poisoning never occurred, in dogs or rabbits, unless circulation was definitely depressed, and the appearance was that of vasomotor failure.

As respiratory stimulants, atropine, caffeine, and camphor were tried; digitalis was used as a circulatory stimulant; and inhalations of CO_2, of 7 to 10 per cent strength, were also used, when respiration was seriously depressed by morphine or heroin.

Atropine stimulated respiration only exceptionally, and the stimulation appeared as an increase in depth but never in rate. As a rule, it either had no effect, or it added to the respiratory and circulatory depression especially if large doses were given—2 to 10 mg. to a dog.

Caffeine was the most reliable and safest stimulant, often increasing both rate and depth, and sometimes bringing back active expiration, especially if it improved the circulation. Large doses did no harm unless they depressed the circulation, when respiratory failure usually followed.

Camphor produced striking stimulation of respiration and circulation in one experiment on a rabbit, in 20 mg. dose, but in all other attempts on rabbits and dogs it either had no effect, or produced circulatory depression and respiratory failure. Its action seemed to depend on improvement of the circulation, and the uncertainty of this effect may explain the uncertain action of camphor as a respiratory stimulant.

Digitalis alone, when it raised blood pressure, sometimes produced definite improvement of respiration. Digitalis and caffeine together proved to be the most reliable form of respiratory stimulation.

Inhalations of CO_2, when expiration was completely passive, often increased the depth but slowed the rate of breathing, and this sometimes actually decreased the minute volume. Frequently CO_2 inhalation was followed by permanent respiratory failure, after a period of gasps of diminishing rate and depth, in dogs and rabbits whose ventilation was adequate before the inhalation. The appearance was that of inhalation of a depressant concentration of CO_2, though the same gas mixture had previously produced only stimulation of respiration.

A study of the clinical records of patients with morphine or opium poisoning in this hospital showed that wherever observations of the circulation were made on patients in a critical condition there were signs of serious circulatory depression, and that as the condition improved under treatment the circulation also improved. One patient died of morphine poisoning, and the pulse could not be felt ten minutes before respiratory failure; pulmonary edema was present, for which the autopsy showed no organic or infectious cause, so that it may have been due to extreme circulatory depression. It is possible that circulatory depression plays a larger part in the dangers of morphine poisoning than is generally realized.

136 (2368)

The types of tetanus bacilli isolated from stools in Peking.

By JOHANNES H. BAUER.

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

We had not seen any confirmation of Tulloch's seriological classification of tetanus bacilli and as we had a number of strains of this organism, isolated from the stools of man, we thought it worth while to see if they could be differentiated into groups by the agglutination reaction. Tulloch not only sent us transplants of his four type cultures but has been most kind in answering our questions and has sent us an impure culture of a strain of tetanus bacilli that did not agglutinate with his type sera. This culture he received from France in 1918 but since no data accompanied it and since it was the only culture that did not agglutinate with his type sera he did not mention it in his work. This culture, which we have called Type V, has interested us very much for, as will be seen in the table, it is a type that we have found most frequently in Peking.

Agglutinating sera were easily prepared by the injection into rabbits of washed bacilli from actively growing 24 hour bouillon cultures. The first injection was subcutaneous and was followed at intervals of a week by three intravenous inoculations. The animals withstood the treatment well and ten days after the last injection their sera agglutinated the type bacillus used in a dilution of 1/6000 or higher. The differentiation of the types is quite striking as the other organisms were not, as a rule, clumped in dilution as low as 1/100.

The results of our tests are given in the table where we have included the findings of Tulloch for comparison.

Results obtained by	Number of tetanus cultures agglutinating in serum type				
	I	II	III	IV	V
Tulloch on cultures from wounds	60	25	35	5	1
Tulloch's with fecal cultures	9	2	1	0	0
Our fecal cultures	3	0	9	0	18

It will be noted that we have confirmed the seriological grouping of tetanus bacilli though we have not, in the small numbers of cultures studied, found Types II or IV. It is interesting that the fifth type that Tulloch found only once is so common here. We have found it not only in human feces but have isolated it from cases of tetanus infections and from the feces of animals. In addition to the types noted above we have twice obtained non motile tetanus bacilli, once from human and the other time from guinea-pig feces. These two strains fail to agglutinate in any of the five type sera but the serum produced by the injection of the human strain agglutinates the guinea-pig as well as the human strain.

While tetanus bacilli can be readily separated by the agglutination reaction we have so far been unable to show that they have any cultural difference except that the Type III bacilli clump spontaneously. This is true not only of Tulloch's culture but of our nine strains. This spontaneous clumping is very constant for this group and makes agglutination tests difficult to read. We have endeavored, by various means, to avoid it, but so far have not been successful.

Tulloch has shown that the antitoxin prepared by the injection of toxin produced by one type of bacillus neutralizes the toxin formed by the other types. We have not studied this point to any extent but have found that commercial antitoxin neutralized the toxin formed by all of our cultures. While he was not able to show conclusively that antibacterial, as well as antitoxin antibodies, are desirable in sera his results point to this and also to the fact that the anti-bacterial antibody is specific for the type bacillus used for its production. This is an exceedingly important point and is worthy of more extended investigation.

137 (2369)

Tetanus carriers in experimental animals.

By CARL TEN BROECK and JOHANNES H. BAUER.

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

The demonstration that human carriers of tetanus bacilli have antitoxin in their blood has opened up a number of problems, which we are attempting to solve on experimental animals. We had first to determine whether carriers could be produced in these animals and if so whether antitoxin would be formed. The results of this preliminary experiment we will report on here and subsequently we hope to give the results of our further studies.

That we would succeed in establishing tetanus bacilli in the digestive tract of guinea pigs was indicated by our finding that 27 per cent of our stock animals were already carriers and that these animals had antitoxin in their serum. On the other hand, we have failed to find a rabbit that carried these organisms nor have we detected tetanus antitoxin in the serum of a number of rabbits examined.

As we planned to use the animals for other experiments a large number of guinea pigs were selected whose stools were free from tetanus spores and whose serum showed no antitoxin. These animals were starved for a day and were then fed a large number of washed spores and bacilli from month old bouillon cultures which were added to their usual food. After the feeding the animals were placed in metal cages which were sterilized from time to time. In order to prevent, as far as possible, infection from the outside all bedding and, with the exception of green stuffs which were washed, all food was autoclaved before it was placed in the cages.

The results of the stool examinations made on these guinea pigs, approximately four months after the feeding of tetanus cultures, are summarized in Table I. We have no reason to believe that the death of any of the quinea pigs was due to tetanus infection, as autopsy showed in the majority of cases a pneumonia. The digestive tract of rabbits has also been infected by feeding but the percentage of positive results is lower than in guinea pigs.

TABLE I.

Type bacillus fed	Number of guinea pigs	Number infected	Number negative	Dead
I	25	16	7	2
II	25	19	6	0
III	25	19	4	2
IV	25	20	4	1

Some of the results obtained from the examination of the blood of these animals are given in Table II. They show that agglutinins, specific for the type fed, appear first but that anti-

TABLE II.

Animal	Feeding	Date of examinations	Tetanus like bacilli in stools	Agglutination titre for			Antitoxin 0.5 cc. serum protects against M. L. D.
				Type I	Type II	Type V	
Rabbit No. 1	On Dec. 27, 28 and 29 fed washed spore of Tetanus Type II	Dec. 12	No	10—	10	10—	0
		Jan. 8	Yes	20—	160	10—	0
		Jan. 20	,,	20—	160	10—	5
		Feb. 2	,,	20—	160	10—	5
		Feb. 17	,,	20—	160	10—	5
		Mar. 5	,,	20—	160	10—	5
		Mar. 29	,,	20—	160	10—	10
		Apr. 29	,,	20—	160	40	25
		May 28	,,	20—	160	40	75
Guinea pig No. 1	On Feb. 20, fed washed spores and bacilli of Tetanus Type II	Feb. 13	No	20	20—	20—	0
		Mar. 28	Yes	20—	80	40	0
		Apr. 28	,,	20—	80	20—	10
		May 26	,,	40	160	20—	40
Guinea pig No. 2	On Feb. 22, fed washed spores and bacilli of Tetanus Type I	Feb. 13	No	20—	20—	20—	0
		Mar. 28	Yes	20	20—	20—	10—
		Apr. 28	,,	80	20—	20—	10—
		May 26	,,	80	20—	20—	40

toxin soon follows and increases in amount. It should be noted that the antitoxin is not specific for type as the toxin used in the tests was produced by Type V bacillus while the animals were carrying Types I or II. The sera of control animals bled at the same time as those that carried the bacilli as well as animals in which tetanus bacilli failed to become established in the digestive tract failed to show either agglutins or antitoxin.

138 (2370)

Schistosomiasis japonica: Egg deposition and the subsequent pathology in the intestines of experimentally infected dogs.

By HENRY EDMUND MELENEY.

[*From the Parasitology Laboratory, Department of Pathology, Peking Union Medical College, Peking, China.*]

Dogs were infected with the cercaria of *Schistosoma japonicum* either by subcutaneous injection or by immersing the dogs in infected water. Eggs were first recovered from the feces of one heavily infected dog on the twenty-seventh day after infection, and from other dogs at various intervals up to about forty days. The dogs were sacrificed at various intervals after the appearance of eggs in the feces, from the twenty-eighth to the seventy-seventh day. Portions of intestine were excised, often while the dog was still alive under ether anesthesia, and were fixed in Zenker's solution or 10 per cent formalin. Blocks were embedded in paraffin and sections were stained with hematoxylin and eosin. From five dogs, killed on the 28th, 33rd, 40th, 63rd and 65th days after infection serial sections were cut from the blocks of tissue which showed the most intense pathology. I wish to emphasize here two features of the pathology which have not been described previously.

1. *The earliest position of deposited eggs.* On the twenty-eighth day after infection nearly all the deposited eggs were in the stroma of the mucosa; a few were in the submucosa. The eggs were in large or small groups containing up to 200 eggs in one group. The eggs of each group usually radiated out into the

mucosa from a single point in the submucosa, and in many cases it could be seen that all the eggs were within distended venules and copillaries. They often extended nearly to the surface epithelium of the mucosa.

Male and female worms, always *in copula,* were found in distended veins in the submucosa. At this early stage there was no hemorrhage into the tissues and practically no tissue reaction about the deposited eggs. It is inferred from these findings that the male and female worms remain together during egg deposition, that the female worm protrudes its head forward into the smaller venules and that it often expels most of the eggs from its uterus within a very short time. (The uterus of this worm, when full, usually contains between 200 and 300 eggs.) The eggs are apparently deposited directly into the vessels of the mucosa, from which point they can reach the intestinal lumen very quickly. This may occur either by means of a rupture of the distended mucosa or by lysis of the tissue due to toxins secreted by the eggs.

2. *Epithelial Proliferation into abscesses in the submucosa.* In sections from the dogs killed 40, 63 and 65 days after infection, abscesses had developed about the eggs in all coats of the intestine, and in each dog there was a tendency for the intestinal epithelium to proliferate into the abscesses in the submucosa. In the 65-day dog some of these abscess cavities were completely lined by intestinal epithelium. In others the epithelium had advanced from the mucosal outlet of the abscess part way around the cavity. The contents of abscesses were being discharged through the lumens of the glands whose epithelium had proliferated inward. No large outlets for submucosal abscesses were seen. This proliferation of epithelium usually occurred where the submucosa was thickened, either because of intestinal rugæ or because of the inflammatory reaction.

The mucosa was often swollen also in such places because of the presence of eggs deposited in it. It is probable that this epithelial proliferation into the submucosa is an important factor in the formation of intestinal papillomas, which are almost constantly found in late stages of schistosomiasis. The thickening of the submucosa and the egg deposits in the mucosa must contribute also to the papilloma formation. Ferguson[1] has described epithelial proliferation into the submucosa in the bladder in human cases of schistosomiasis hematobia, and considers it a factor in papilloma formation in that viscus.

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[1] Ferguson, A. R., *J. Path. and Bact.*, 1911, xvi, 76.

ABSTRACTS OF COMMUNICATIONS.

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139 (2371)

The possible rôle of constant bioelectric currents in growth.

By E. J. LUND.

[*From the Laboratory of General Physiology, University of Minnesota, Minneapolis, Minn.*]

In a previous note[1] it was stated that the threshold density of an electric current necessary for inhibition of growth in the ends of Obelia internodes turned toward the cathode was very nearly equal to 66 microamperes per square millimeter.

This threshold inhibition is reversible.[2] For orientation of the axis of growth a threshold current density of only 6 to 13 microamperes per square millimeter of cross section is necessary. This is therefore only about one-tenth as great as the current density threshold for inhibition. We may translate this result into its equivalent of threshold of applied difference of electric potential.

The living tissue of the stem and pieces of stem in Obelia have also been shown to be the seat of a relatively constant electric potential.[3] More recently this potential has been measured by the use of a suitable type of electrode.[4] The results show that a difference of potential exists between the inside and outside of the ecto-endoderm layer. The outside surface of the ectoderm being electro-positive to the inside surface of the endoderm. Similar potential differences are well known to occur in the skin of the frog, intestine and some other epithelial structures. The magnitude of the inherent potential in the ecto-endoderm of Obelia is greater in the apical growing point of the main stem of the colony than in the middle, and somewhat lower regions of the same stem. The same statement applies to growing branches of the colony. The result of this difference in the magnitude of the electric potentials across the ecto-endoderm in apical and more basal parts of the stem, is that when the outer surfaces of the apical and basal regions of the stem are connected to a galvanometer, a current flows in the galvanometer circuit from apical to basal parts of the isolated stem.

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[1] Lund, E. J., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 127.

[2] Lund, E. J., *J. Exp. Zool.*, 1923, xxxvii, 69.

[3] Lund, E. J., *J. Exp. Zool.*, 1922, xxxvi, 477.

[4] Lund, E. J., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 128.

Now, this inherent current has certain important characteristics. (1) It is relatively constant, and therefore differs from the intermittent bioelectric currents of muscle, nerve, and electric organs of some fish. (2) It is definitely directed, *e. g.,* from inside surface of endoderm to outside surface of ectoderm. (3) Its magnitude across the ecto-endoderm has been found in actual measurements to vary approximately from 0.2 to 10 millevolts in apical pieces of the main stems of different colonies. It can be shown that this range of values is very nearly the same as the calculated range of applied threshold potentials for inhibition of growth toward the cathode. But the direction of the inherent fall of potential at the point of inhibition of growth in an internode is opposite to the direction of the applied potential.

These facts lead to the simple conclusion that inhibition of growth toward the cathode is associated with a diminution or complete neutralization of the inherent difference of potential across the ecto-endoderm by the applied E. M. F. at the point of growth.

Now since the threshold value of the applied potential for orientation of growth is only about one-tenth as great as the threshold of the applied potential for inhibition, and since the latter is nearly the same as the inherent potential across the ecto-endoderm, it follows that the inherent potential difference is approximately ten times greater than would be necessary for a perceptible orientation effect upon the growing tissue, if the inherent potential difference were to be suitably applied to the growing tissue.

It will be observed that the three characteristics named in (1), (2) and (3) above are specifically the same as those of the applied electric current which is necessary for the control of growth in Obelia internodes. In view of the above facts we are forced to a consideration of the possible rôle of such inherent bioelectric currents in the phenomena of inhibition and orientation of growth processes.

In brief the facts suggest a possible new type of mechanism for cell correlation which could conceivably be operative in the early development of animals and plants where neither a visible structural nervous system exists, nor any known specialized mechanism for the transport of chemical substances (hormones).

The detailed experimental facts upon which the statements in this note are based will be and have been in part presented in the Journal of Experimental Zoology.

140 (2372)

Experiments with an active extract of parathyroid.

By E. O. ELLINGSON, A. W. BELL and ADOLPH M. HANSON

(by invitation).

[*From the Department of Chemistry, St. Olaf College, Northfield, Minn., the Department of Pharmacology, Emroy University, Atlanta, Ga., and Faribault, Minn.*]

Recently, H. W. C. Vines has shown that calcium in normal unclotted blood is present in two forms. He found that approximately one-third of the calcium is present in a combined or non-ionized state, while two-thirds are in the ionized form. He also observed that chronic septic infections do not heal rapidly unless the ionic calcium is increased. Vines observed that the administration of dried parathyroid increased the relative ionic calcium and lead to rapid healing of ulcers.

The present authors have also observed similar effects, using an extract of parathyroid made in the following manner: 30 gm. of finely divided ox gland are boiled in 500 cc. of dilute hydrochloric acid (10 parts per thousand), cooled, filtered, and then made up to 500 cc. again. This extract which we know as hydrochloric X seemingly contains the active principle of the parathyroid. Dogs which had been completely parathyroidectomized and which were showing symptoms of tetany, recovered. Sometimes on injection, the symptoms increased at first before recovery took place.

This extract we have also found beneficial in the treatment of chronic inflammations such as gastric, duodenal, and varicose ulcers as well as cystitis or osteomyelitis.

From this hydrochloric X the active constituent may be precipitated by phosphotungstic acid, but we have not been able to redissolve the active constituent from this precipitate.

141 (2373)

The respiratory wave in arterial blood pressure.

By MAURICE B. VISSCHER and ALICE RUPP (by invitation).

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*]

The problem of the causation of the respiratory variations in blood pressure is an old one, but one that has resisted solution surprisingly long. Examination of the literature on the subject shows that diametrically opposite observations have been made upon the relation between the phases of respiration and the rise and fall of blood pressure. About half of the workers have found an inspiratory rise, and half an inspiratory fall.

In examining the factors heretofore mentioned in explaining the phenomenon it is apparent that the possibility that factors tending to change blood pressure might not be immediately effective, has been neglected. In other words, the possibility that the effects of inspiration might show up in expiration, has been neglected.

In experiments in which the inferior vena cava, the superior vena cava and the azygos vein were clamped separately and in combination, it was found that there was absolutely no sign of these changes on the arterial pressure trace for a period, on the average, of three heart beats. Very evidently there is a latent period of three heart beats in this process.

The changes in arterial pressure were similarly recorded when variations were artificially produced in the thoracic cavity. Similar latent periods were observed.

These experiments were repeated upon animals with denervated hearts and the same observations made, excluding a nervous factor.

It is evident that the effects of inspiration may show up entirely in expiration and the effects of expiration in the succeeding inspiration. The heart-respiration ratio determines in what phase of respiration the various changes will appear.

Many published arterial pressure tracings have been examined and it has been found that this explanation is quite valid in interpreting them.

142 (2374)

Production of rickets and osteoporosis on diets of purified food substances.

By J. F. McCLENDON.

[*From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis, Minn.*]

In experiments on rickets, osteoporosis and xerophthalmia by my students and myself, white rats belonging to litters 1-150 were fed diets 1-467. In most of these white flour was an ingredient but some were made of purified food substances. It was found that a group of vitamins known as Vitamin A were all contained in the cod liver oil used; that commercially dehydrated spinach, or spinach dried in the laboratory at a low temperature, contained a growth promoting Vitamin A, and prevented xerophthalmia, but had no detectable antirachitic effect. Its effect in preventing osteoporosis was extremely small, and since the spinach contained calcium it was very difficult to decide whether the effect was due to calcium or due to vitamin. Extracts made from this spinach also failed to show antirachitic and antiosteoporotic effects. Vitamin B was derived from bakers' yeast. In most of these series of experiments, the antirachitic vitamin was kept low and constant while the calcium and phosphorus were varied. Results confirm those of Sherman and Pappenheimer as well as those of Osborne, Mendel and Park. It has not been possible so far, however, to show that rickets depended on any ratio of calcium to phosphorus, the most striking result being that low calcium produces osteoporosis and low phosphorus produces rickets.

Diagnosis of rickets was made by Dr. C. M. Jackson[1] in the earlier experiments. By comparing the diagnosis made by Dr. Jackson on gross and microscopic pathology with x-rays taken the same day, a basis of interpretation was made of x-rays, and x-rays were used for diagnosis in the later experiments. It is not possible to record all these experiments but the following is given as an example. Ca was added as $CaSO_4$ and P as $KH_2\,PO_4$. The weights in each diet were planned to add up to about 100.

	Osteoporosis Mild Rickets	Osteoporosis	Osteoporosis	Mild Rickets	Normal	Normal
Lactalbumin	10	10	10	10	10	10
Vitamin B	5	5	5	5	5	5
Antiophthalmic vitamin	1	1	1	1	1	1
P	.2	.35	.5	.2	.3	.5
Ca	.2	.2	.2	.8	.8	.8
Other salts	2	2	2	2	2	2
Starch	62	61.7	61.4	60	59.7	59.4
Triolein	20	20	20	20	20	20

In the above table it is noticed that osteoporosis occurred on low calcium diets and mild rickets occurred on low phosphorus diets. Unfortunately, these diets contained a small amount of antirachitic vitamin but this was constant for all of the diets recorded in the table.

The rats were kept in a north room with glass windows closed all of the time. The mothers were fed on Steenbock's rat diet with the substitution of fresh milk by whole milk powder. The young, when 21 days old, weighed about 30 gm. and were placed on the special diets and allowed to remain for 30 days.

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[1] Jackson and Carleton, *Am. J. Physiol.*, 1923, lxv, 1.

143 (2375)

The effect of the surface tension of the medium upon bacterial toxins.

By W. P. LARSON and EDMOND NELSON.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

Tetanus toxin, diphtheritic toxin, botulinus toxin and endotoxin, obtained from the actinomyces gypsoides, were treated with castor oil soap to determine its effect upon these various toxins. Tetanus toxin and diphtheritic toxin were found to be completely detoxified when sufficient castor oil soap was added to reduce the surface tension below forty dynes. Guinea pigs treated with one hundred lethal doses of the respective toxins, with the soap, failed to develop symptoms.

Botulinus toxin appears to be more resistant to soap than the other two soluble toxins. This observation offers a possible explanation of the fact that neither diphtheritic nor tetanus toxins are absorbed in toxic form by way of the digestive tract; while botulinus toxin is readily absorbed by the digestive organs. The contents of the intestinal canal having a low surface tension, because of the bile and soaps present, probably destroy tetanus and diphtheritic toxins, but not botulinus toxin.

Nelson and Henrici[1] have shown that the actinomyces gypsoides, an organism very pathogenic for guinea pigs, possesses a potent endotoxin which kills rabbits and guinea pigs regularly. When shaken in a two per cent solution of castor oil soap, so as to effect a thorough "wetting", this organism loses its pathogenicity, and its endotoxin is also completely destroyed.

Whether or not the effect of soap on bacterial toxins is entirely a surface effect, or is due, in part, to some other property of the soap, cannot be determined definitely at this time.

Control experiments have convinced us that the action of the soap upon the bacterial toxins is not due to the low hydrogen ion. The effect of soap on the antigenic properties of bacterial toxins will be discussed in a future paper.

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[1] Nelson, E., and Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 351.

ABSTRACTS OF COMMUNICATIONS.

Western New York Branch

Tenth Meeting.

Rochester, New York, February 16, 1924.

144 (2376)

The rôle of surface tension and potential difference in the stability of a diphtheria-like bacillus.

By RALPH R. MELLON, W. S. HASTINGS and C. ANASTASIA.

[*From the Highland Hospital, Rochester, N. Y.*]

It has been found possible to emulsify a highly cohesive and agglutinable strain with M/50 sodium oleate, which concentration was less than for any other of a number of salts used, which included M/16 MgCl, M/8 Na Citrate, M/2 NaCl, copper acetate and $CaCl_2$, which are given in the order of their emulsifying efficiency.

With threshold concentrations of certain of the emulsifying salts the organism spreads suddenly over the surface of the liquid. The phenomenon is well recognized to be one of surface tension depression.

It is probable that the emulsifying action of the oleate is brought about by its ability to diminish the abruptness at the interfacial boundary between the bacterium and the solution. This view accords with the fact that COONa radical of the oleate is truly soluble in the water, and the hydrocarbon radical is not. The obvious lipoidal nature of the wall of the bacterium in question makes probable the adsorption of the oleate anion; in fact, repeated washing restores the flocculability. These conditions make for diminution of abruptness at the interfacial boundary which always produces a marked lowering of surface tension. Measurements of potential difference indicate the recognized importance of this factor also, but in the more dilute concentrations of salts.

A closely related strain emulsifiable in NaCl was precipitated by $CaCl_2$ and an ion antagonism was shown between the Na and Ca cations in a ratio of 80:1. The effect was not permanent. The ratio is of the same order of magnitude as in oil-water and water-oil emulsions and suggests a similar lipoid arrangement in the membrane of the organism.

145 (2377)

A study of the reaction of normal human subjects to intravenous injections of insulin.

By AARON BODANSKY and SUTHERLAND SIMPSON.

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

Two-tenths of a cc. of insulin were injected intravenously. Blood samples were withdrawn at frequent intervals, and sugar determinations were carried out on the whole blood.

The blood sugar decreases rapidly and at a uniform rate. The minimum sugar value is indicated at about 18 minutes after the injection. The sugar content of the blood then rises, at a slower rate, reaching the initial value within about one hour after the injection.

Peculiarities were observed in the reaction of overweight subjects. The insulin hypoglycemia is not as intense as in a lean subject of the same weight, and recovery from it seems slower. While this phenomenon apparently corroborates older views on the subject of "fat and glycogen antagonism", it is being investigated further, and other experiments are being undertaken on the relationship between fat and glycogen formation.

It is suggested that the varying degrees of adiposity, as such, underlie the differences in the reaction to insulin of animals used in its assay.

The experiments were carried out, in part, under a grant from the Heckscher Research Foundation.

ABSTRACTS OF COMMUNICATIONS.

Pacific Coast Branch

Fortieth Meeting.

San Francisco, California, February 13, 1924.

146 (2378)

On the mode of union of certain proteins with acids and bases.

By D. M. GREENBERG and CARL L. A. SCHMIDT.

[*From the Department of Biochemistry and Pharmacology, University of California, Berkeley, Calif.*]

Considerable experimental evidence has in recent years been brought forth which indicates that the union of proteins with acids and bases takes place by chemical combination.[1] It has been suggested by Robertson[2] that the -COHN- groups in the protein molecule are responsible for the acid- and base-combining capacity but this hypothesis has been recently questioned by ourselves.[3] Kossel and Cameron[4] pointed out many years ago that the acid-combining capacity of salmin is equal to the combining capacity of the guanidine groups in the arginine, and Bracewell[5] has carried this idea further by showing in a number of proteins a close agreement between the acid-combining capacity and the sum of the free amino nitrogen and the amino group of arginine which does not react with HNO_2. The recent work of Hitchcock[6] in which he determined the acid-combining capacity of gelatin and of deaminized gelatin affords an opportunity for checking this hypothesis and our calculations which are given in Table I show an excellent agreement between the calculated and the experimental results.

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[1] Loeb, J., *Proteins and the Theory of Colloidal Behavior*, New York, 1922.

[2] Robertson, T. B., *The Physical Chemistry of the Proteins*, p. 24.

[3] Greenberg, D. M., and Schmidt, C. L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 197.

[4] Kossel, A., and Cameron, A. T., *Z. physiol. Chem.*, 1912, lxxvi, 457.

[5] Bracewell, R. S., *J. Am. Chem. Soc.*, 1919, xli, 1511.

[6] Hitchcock, D. I., *J. Gen. Physiol.*, 1923, vi, 95.

TABLE I.

Combining Power of Gelatin for Acids.

Amino Acids	Per cent of total N	Mols N which combine with acid	Mols HCl which should be bound	Mols HCl which are bound (based on Hitchcock's data)
Arginine nitrogen[10]	14.7	47×10^{-5}	47×10^{-5}	45×10^{-5}
Lysine nitrogen[10]	6.3	41×10^{-5}	41×10^{-5}	44×10^{-5}

The discovery of β-hydroxy-glutamic acid in certain proteins by Dakin[7] and the more recent work by Harris[8] showing that tyrosine must be regarded as a dibasic acid has given us data which we have used in attempting to determine the seat of the base-combining power in the protein molecule. The early experiments of Osborne and his collaborators indicate that the second carboxyl group of the dibasic amino acids does not exist free in certain of the vegetable proteins but is combined as the acid amide. This view is probably not strictly true, especially if the content of β-hydroxy-glutamic acid is considered. In the instance of casein which is predominantly acid the discrepancy between the amide nitrogen and the content of dicarboxylic acids is considerable. The experiments of Osborne and Nolan[9] in which they found a direct relationship between the increase in base-combining power of certain proteins and the ammonia which was split off by mild hydrolysis strongly points to the dicarboxylic acids as the seat of the base-combining power of the protein molecule.

Accurate data upon which to base calculations are available only for several proteins and while our data (Table II) do not

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[7] Dakin, H. D., *Biochem. J.*, 1918, xii, 290.

[8] Harris, L. J., *Proc. Roy. Soc.*, 1923, xcv, 440.

[9] Osborne, T. B., and Nolan, O. L., *J. Biol. Chem.*, 1920, xliii, 311.

[10] Dakin, H. D., *J. Biol. Chem.*, 1920, xliv, 499.

TABLE II.

Base-combining Power of Casein.

Amino Acids	Mol. Wt.	Per cent	Mols of NaOH which should be bound (per gm. of protein)
Glutamic[7]	147	21	143×10^{-5}
Aspartic[7]	133	3.8	26×10^{-5}
β-hydroxy-glutamic[7]	163	10.5	64×10^{-5}
Tyrosine[11]	181	5.8	32×10^{-5}
			Total 265×10^{-5}
Less amide nitrogen[11]	14	1.5	107×10^{-5}

Mols NaOH which should be bound, 158×10^{-5}.
Mols NaOH which one gm. of casein binds at pH 12, 160×10^{-5}.

Base-combining Power of Gelatin.

Amino Acids	Mol. Wt.	Per cent	Mols of NaOH which should be bound (per gm. of protein)
Aspartic[10]	133	3.4	26×10^{-5}
Glutamic[10]	147	5.8	39×10^{-5}
			Total 65×10^{-5}
Less amide nitrogen[10]	17	0.4	23×10^{-5}

Mols NaOH which should be bound, 42×10^{-5}.
Mols NaOH which one gm. of gelatin binds at pH 11, 60×10^{-5}.

Base-combining Power of Gliadin.

Amino Acids	Mol. Wt.	Per cent	Mols of NaOH which should be bound (per gm. of protein)
Glutamic[12]	147	43.7	297×10^{-5}
Aspartic[12]	133	0.2	—
Tyrosine[13]	181	5.1	28×10^{-5}
β-hydroxy-glutamic[14]	163	2.4	15×10^{-5}
			Total 340×10^{-5}
Less amide nitrogen[15]	17	5.2	306×10^{-5}

Mols NaOH which should be bound, 34×10^{-5}.
Mols NaOH which one gm. gliadin binds at pH 11, 30×10^{-5}.

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[11] Dunn, M. S., and Lewis, H. B., *J. Biol. Chem.*, 1921, xlix, 327.

[12] Osborne, T. B., and Guest, H. H., *J. Biol. Chem.*, 1911, ix, 425.

[13] Cross, R. J., and Swain, R. E., *J. Ind. Eng. Chem.*, 1924, xvi, 49.

[14] Dakin, H. D., *Biochem. J.*, 1919, xiii, 398.

[15] Osborne, T. B., Van Slyke, D. D., Leavenworth, C. S., and Vinograd, M., *J. Biol. Chem.*, 1915, xxii, 259.

show absolute agreement between the amount of alkali which was experimentally found to be taken up by the proteins and that which was calculated on the basis of the free dibasic acid groups, the values nevertheless are of the same magnitude and indicate at least from the qualitative standpoint that there is a distinct relationship between the alkali-combining power of these proteins and their content of free dibasic acid groups. The greatest discrepancy is found in gelatin. This, however, is not serious especially since it is very probable that in the estimation of small amounts of aspartic and of glutamic acid considerable experimental error enters.

Our method of estimating the base-combining power of the proteins was carried out according to the procedure which has previously been used by Tague[16] for amino acids and by Loeb and Hitchcock for proteins. On account of the logarithmic increase in pH on addition of alkali the method is not capable of a very high degree of accuracy at high alkalinity.

147 (2379)

Reactions of the urinary bladder in canine anaphylaxis.

By W. H. MANWARING, V. M. HOSEPIAN, J. R. ENRIGHT and DOROTHY F. PORTER.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, Calif.*]

As an index to possible smooth muscle reactions, we have studied the pressure changes in the urinary bladder during canine anaphylactic shock, with parallel tracings of the changes in the arterial blood pressure. The dogs were sensitized to horse serum by Weil's method.[1] They were tested by intravenous injections of 0.5 to 2.0 cc. horse serum per kg. of body weight, about twenty-one days after the final sensitizing dose. The intracystic pressure was recorded by means of a glass catheter (perineal incision, male dogs). The abdomen was opened to avoid errors from changes in intraabdominal pressure. The following cystic reactions have thus far been recorded:

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[16] Tague, E. L., *J. Am. Chem. Soc.*, 1920, xlii, 173.

[1] Weil, R., *J. Immunol.*, 1923, viii, 233.

1. *Typical Anaphylactic Shock.* Arterial blood pressure falls precipitously to about 35 mm. Hg by the end of 45 seconds, gradually decreasing to about 25 mm. Hg by the end of 90 seconds. Recovery usually begins about the twelfth minute, the blood pressure being restored to normal in from 60 to 90 minutes. No recordable change in the intracystic pressure during the first 45 to 75 seconds. The intracystic pressure then gradually increases, usually reaching a maximum of from 25 to 50 mm. Hg by the end of two and a half minutes. Recovery immediately sets in, the intracystic pressure usually being reduced to normal by the seventh minute. No reactions were observed in normal controls.

Assuming that the cystic reactions thus recorded are reliable indices to smooth muscle reactions in other parts of the body, one must conclude that in typical canine anaphylactic shock the smooth muscle structures are not thrown into contraction until the shock is fully established, as determined by the fall in arterial blood pressure. The smooth muscle reactions, therefore, apparently play no rôle in the initiation of the shock. They also apparently have little or no effect on the duration or severity of the shock, as determined by the changes in arterial blood pressure.

2. *Fatal Anaphylaxis.* We have had one example in this series of the rapidly fatal type of canine anaphylaxis previously reported.[2] In this dog, increase in the intracystic pressure began about the 45th second, the pressure reaching a maximum of 30 mm. Hg by the 75th second. The intracystic pressure was maintained at this maximum, practically unchanged till the death of the animal, death taking place in nine and one-half minutes. The thorax of this dog was immediately opened. The lungs remained inflated, were of a rubber-like consistency, and could not be collapsed on moderate pressure. Histological findings will be reported later.

In the infrequent, rapidly fatal type of canine anaphylaxis, therefore, smooth muscle reactions apparently become the dominant factor during the later stages of the shock, and are apparently the immediate cause of death. Even in this type of shock, however, the smooth muscle reactions apparently play no rôle in initiating the shock, as determined by the fall in arterial blood pressure.

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[2] Manwaring, W. H., Chilcote, R. C., and Hosepian, V. M., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 274.

148 (2380)

Convulsions resulting from fluid administration following sucrose injections and water abstinence.

By LOIS LOCKARD MacKAY and EATON M. MacKAY (by invitation).

[From the Department of Medicine of the Stanford University Medical School, San Francisco, Calif.]

Convulsions and immediate death were found to result from the administration of fluid to rabbits which had been dehydrated by intravenous injections of sucrose 5 to 10 days previously.

These animals had been used to determine the effect of water abstinence and increased water loss on the excretion of urea and the blood urea concentration. They were kept without food or water and bled 1 to 3 times daily throughout the experiment. Extremely high blood urea concentrations (100 to 450 mg. per 100 cc.) were reached in every animal and fluid ingestion resulted in a very much higher blood urea concentration.

Water to the amount of 200 cc. was administered to a rabbit which had undergone simple water abstinence with negative results. Convulsions were obtained in those rabbits which had received sucrose after the administration of 100 to 200 cc. of distilled water or 0.9 per cent sodium chloride.

The severity of the albuminuria and the height of the blood urea concentration was greatest in the rabbits which had been dehydrated previous to water abstinence by intravenous sucrose injections. Since it was in these only that convulsions were produced it would seem that some damage produced by sucrose was a factor. The cause of these convulsions and their association with kidney damage is being further investigated.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF COMMUNICATIONS.

One hundred and thirty-eighth meeting.

University and Bellevue Hospital Medical College, March 19, 1924.
President Jackson in the chair.

149 (2381)

A new and delicate biological method of detecting carbon monoxide in blood.

By DAVID I. MACHT, S. S. BLACKMAN JR., and MARGARET SWIGART.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

In connection with a phytopharmacological study of menstrual toxin by Macht and Lubin published elsewhere, the authors had occasion to examine the effects of various specimens of pathological blood on the growth of plants and found that some of them were quite toxic for plant protoplasm. Inasmuch as carbon monoxide forms a firm compound with hemoglobin, it was thought possible that such blood might exert a poisonous effect on phytopharmacological preparations. Accordingly experiments were made with carbon monoxide blood of various animals on the growth of seedlings of *Lupinus albus* by methods described elsewhere. Series of seedlings were suspended in normal Shive solutions on the one hand and in solutions of normal blood and of monoxide blood on the other hand. It was found that carbon monoxide blood was extremely toxic for plant protoplasm and furthermore that this toxicity, biologically speaking, was proportional to the concentration of carbon monoxide in the blood. The sensitiveness of *Lupinus albus* roots to carbon monoxide blood is illustrated by the following data. Some defibrinated pig's blood was saturated with pure carbon monoxide, and solutions of this blood were made in a mixture of equal parts of Shive and distilled water. It was found that a 1 per cent solution of blood produced a very marked inhibition and even 0.1 per cent solution of the blood also produced a distinct and easily measurable inhibition in the elongation of the roots. Further experiments indicated that solutions of monoxide blood as dilute as 0.01 per cent produced in sensitive preparations a definite inhibition in the growth of seedlings as compared with the growth of a similar series of seedlings immersed in a similar solution of normal pig's blood.

The authors have plotted curves showing a simple relationship between the concentrations of carbon monoxide hemoglobin and the toxicity of various solutions for plants. The greatest usefulness of the present method in studying carbon monoxide blood is for comparative purposes, such as in the studies by Macht and collaborators on the treatment of monoxide poisoning described in the following communication. Experiments with pure hemoglobin and carbon monoxide hemoglobin gave similar results as were obtained with blood. The data furnished by the plant experiments agreed well with quantitative chemical analysis, but the phytopharmacological method has been found by the authors for practical purposes to be more delicate and more convenient. Even dry blood can be studied in the same way; such blood being dissolved in distilled water and the solutions of hemoglobin thus obtained treated as above. The plant method of studying monoxide poisoning would speak for a specific toxicity of carbon monoxide aside from the asphyxia produced by it, a mooted question which cannot be satisfactorily settled by animal experiments. By the present method the presence of carbon monoxide in plasma can also be demonstrated. Poisoning with illuminating gas has also been studied by the authors. The blood obtained in such cases is even more toxic for plants than pure monoxide blood on account of the presence of ethylene gas in solution, which gas, as is well known, is very toxic for plants. This announcement is a preliminary communication and complete data of the present and further work will be published in due time.

150 (2382)

An experimental contribution to the treatment of carbon monoxide poisoning.

By DAVID I. MACHT, S. S. BLACKMAN JR., and E. B. KELLY.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

Macht and Teagarden in a study concerning the effects of light on the toxicity of quinine and quinidine sulfates have found that the activity of these drugs is potentiated by exposure to ultraviolet rays. In connection with these experiments it occurred to one of the authors that such radiations might possibly influence the toxicity of carbon monoxide for animals by hastening the dissociation of carbon monoxide from the hemoglobin with which it is firmly combined. A few preliminary experiments performed on rats seemed to corroborate this supposition. It was found in a series of rats, all of which were poisoned to the same degree with carbon monoxide, that those animals which were exposed to direct sunlight recovered more quickly than other rats similarly poisoned which were kept at the same temperature in a dark place. An extensive investigation was accordingly begun on the effects of light and more particularly of ultraviolet radiations on the toxicity of carbon monoxide. Experiments were made on shed blood, on the one hand, and "clinically" on various animals such as mice, rats, rabbits and dogs on the other hand. It was found that animals which were poisoned with gas and were radiated with ultraviolet rays recovered much more quickly and completely than similar animals poisoned to the same degree and which were kept at the same temperature in the dark. Comparative studies of blood on the two conditions showed that the break-up or dissociation of carbon monoxide hemoglobin took place much more rapidly under quartz-lamp radiation than normally. In making such comparative studies the phytopharmacological method of testing for carbon monoxide hemoglobin was found to be especially convenient and useful. The radiations employed in the present experiments were obtained from the Hanovia "Alpine Sun" quartz mercury vapor lamp. It was further found that a slight acceleration in the degree of dissociation of carbon monoxide hemoglobin can be produced by increase of temperature. The marked break-up of the monoxide hemoglobin through ultraviolet radiation was shown, however, to be entirely independent of the heat waves, inasmuch as in all experiments the effects of radiations on animals were compared with control animals kept in a dark room at the same temperature.

Experiments have been undertaken to determine the relative value of radiations of various wave lengths obtained from the mercury vapor lamp. Furthermore, the finer mechanism of action of ultraviolet rays on carbon monoxide blood has been analyzed by physico-chemical methods by the senior author with the collaboration of Dr. Arthur Grollman of the Chemical Department of this University. The penetration of various rays through the skin and other animal tissues is also being investigated. Full data will be published in due time.

151 (2383)

The effect of ultraviolet, x-ray, and radium radiations on the phytopharmacological reactions of normal blood.

By DAVID I. MACHT and EBEN C. HILL.

[*From the Pharmacological and Anatomical Laboratories of the Johns Hopkins University, Baltimore, Md.*]

Phytopharmacological investigations by Macht and various collaborators have brought out the interesting fact that living plant tissues are often more sensitive to poisons and metabolic products of animal origin than living animal tissues are to the same substances. This was found to be especially true of normal as compared with pathological blood specimens, as, for instance, in case of menstrual blood, described by Macht and Lubin elsewhere. It occurred to the authors that possibly by the use of phytopharmacological test preparations certain changes in normal blood might be detected which are not demonstrable by the use of animal tissues or preparations. In the present investigation such a study was made of normal defibrinated pig's blood under ordinary conditions, on the one hand, and after radiation with rays of various wave length on the other hand.

The tests were performed by measuring the growth of living seedlings of the plant *Lupinus albus* immersed in definite nutrient solution with and without admixture of one per cent of blood. Specimens of pig's blood were exposed to the action of ultraviolet rays for periods varying from 10 to 30 minutes, the radiations being performed by means of the Hanovia "Alpine Sun" mercury vapor quartz lamp. Other specimens of blood were x-rayed by one of the authors with broken doses from Coolidge and nitrogen gas tubes for periods varying from 1 to 3 minutes. Finally a few experiments were made with blood treated for one hour with radium emanation of small dosage.

The results obtained were interesting. The toxicity of blood for *Lupinus* seedlings after ultraviolet radiation was unaffected and in some cases was lightly diminished. In case of Roentgen rays the toxicity of blood after radiation was distinctly increased, and a difference was noted between specimens radiated with the two kinds of tubes. Radiation with the Coolidge tube rendered the blood much more toxic for the plants than radiation with the gas tube. The few experiments performed with blood exposed to radium emanation gave results practically the same as those obtained with Roentgen rays; that is, the blood was rendered more toxic for the phytopharmacological preparations. Furthermore, after treatment with radium the blood exhibited a distinct hemolysis.

152 (2384)

Culture filtrates of hemolytic streptococci from scarlet fever: Intracutaneous reactions in test animals.

By A. W. WILLIAMS, H. D. HUSSEY and E. J. BANZHAF.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

A number of substances poisonous for animal cells have been described as appearing in filtrates of young cultures of hemolytic streptococci. The presence of hemolysin (Besredka, 1901) and leucocidin (Ruediger, 1905) with the production of an antiserum for the latter and not for the former has been many times corroborated. The presence of a specific exotoxin (Clark and Felton, 1918, Havens and Taylor, 1921) has been denied by Zinsser, Parker and Kuttner (1921), who state that they find in these young culture filtures only non-specific low grade poisons (called "X substances" by them) that do not stimulate the production of antiserums. For all this work, the animal tests of the poisonous products were made only by way of the veins, the subcutis or the peritoneal cavity. Zinsser reports only intravenous inoculations. The latest and most suggestive announcement has just been made by the Dicks.[1] They have a toxic filtrate obtained from cultures of hemolytic streptococci from scarlet fever, that gives an *intracutaneous* reaction in people presumably susceptible to scarlet fever, that is neutralized by serum from scarlet fever convalescents, and that gives no intracutaneous reaction in scarlet fever convalescents.

The Dicks kindly sent us some of their toxic filtrate, and Dr. Zingher in our laboratory has fully corroborated the Dicks' use of their test in humans. We have obtained similar toxic filtrates from the hemolytic streptococci we have isolated from scarlet fever cases. According to Dr. Zingher's tests, these act in humans as does the filtrate obtained from the Dicks. Our methods of growth will be given later. As the Dicks have not yet published their culture methods in detail, we cannot say how ours agree. We have found that the intracutaneous injection of these filtrates into young depilated rabbits gives a very clear cut reaction and that the filtrates are neutralized by serum from convalescent scarlet fever patients. The reaction in guinea pigs is not as clear cut as in the rabbits.

The Dicks' toxic substance is in a four-day culture filtrate. These investigators have not yet published attempts to work out the curve of production. We have found that so far our own filtrates give the strongest and most lasting reaction in rabbits at about the end of the second day of growth, after which there is a moderate drop to the end of the fourth day which is the latest we have tested them. This curve corresponds to that reported by Clark and Felton for the appearance of their toxic filtrate and, according to the published reports, leaves behind by many hours the curves of the production of hemolysin, of leucocydin and of the X substances of Zinsser, et al.

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[1] Dick, G. F. and G. H., *J. Am. Med. Assoc.*, 1924, lxxxii, 265.

In the tests in rabbits we have been able to show so far that while convalescent scarlet fever serum seems to neutralize the filtrates of the growth of the hemolytic streptococci from scarlet fever, as shown by the intracutaneous reaction, it has not yet neutralized toxic filtrates obtained in the same way from *Staphylococcus pyogenes* or from *Escherichia coli.* Neither has it neutralized a toxic filtrate obtained from a hemolytic streptococcus from endocarditis while it has neutralized one (strain F) isolated from a wound infection, which we included by absorption of agglutinins in our agglutinative group of hemolytic streptococci obtained from scarlet fever. Young rabbits (about 1500 gm.) of the type giving a good skin reaction with vaccine virus and depilated as we depilate for that test seem to be especially suitable for this test and in the many questions of interest that are coming up in connection with this work they promise to be a great help.

153 (2385)

Results obtained with the Dick test in normal individuals and in acute and convalescent cases of scarlet fever.

By ABRAHAM ZINGHER.

[*From the Bureau of Laboratories, Department of Health and the Willard Parker Hospital, New York City.*]

Studies have been made with the Dick test among normal individuals along lines similar to those carried out with the Schick test. The susceptibility by age groups from birth to adult life has been investigated and found to correspond closely to the percentage susceptibility to diphtheria, as shown by the Schick test. Comparative studies have been made with the reaction on mothers and their offspring. The results indicate that the reactions are similar in the mother and offspring during the first six months of life. The antibodies are transmitted through the placenta and persist in most infants for a period of about six to nine months. The blood sera of individuals who gave positive or negative Dick reactions have been studied for their properties in causing the extinction test of Schultz-Charlton in a fresh scarlet fever rash; also for their power to neutralize the test fluid for the Dick reaction.

The blood serum of a person giving a "negative" or a "pseudo" Dick reaction causes blanching of the rash and neutralizes the test toxin; that of a "positive or a combined" reaction causes no blanching of the rash and does not neutralize the test toxin. The Dick test is positive as a rule during the first two days of scarlet fever but becomes less positive toward the end of seven days and negative toward the end of ten to fifteen days, when the antitoxic properties begin to appear in the patient's serum. This can be shown by its power to blanch the scarlet fever rash and neutralize the Dick test toxin. The neutralization of the test toxin is a more delicate index of the presence of antibodies in a serum than its property to cause blanching of a scarlet fever rash. The blanching or Schultz-Charlton test is a fairly crude phenomenon and depends on too many variable factors such as the character of the rash and its duration before the serum injection, the accuracy of the intradermal injection, etc. In spite of these drawbacks the test is of some value in showing whether a suspicious rash may be scarlet fever or not, especially when the test is made during the early stage of the disease.

Quantitative studies have been made with increasing concentrations of the test fluid to determine the approximate amount of antibodies that develop during the convalescence from scarlet fever, the antibody content of the serum of normal negative Dick reactors and of the antitoxic horse serum prepared by Dochez. The antibody content is determined by one of two methods: (1) By testing the individual directly with increasing concentrations of the test toxin, beginning as Dick recommended with a dilution of 1:1000 or indirectly; (2) By diluting equal volumes of the test toxin (1:500) with increasing dilutions of the serum, undiluted, 50, 33, 25, 20, 10, 5, 2.5 and 1 per cent, allowing the two mixtures to remain preferably at room temperature for 30 minutes and then injecting either all of these test mixtures or alternating ones into an individual who has shown a good positive reaction with the 1:1000 dilution. If the standard toxin dilution is 1:500, then the serum is added to a toxin dilution of 1:250, so that the ultimate dilution of the toxin in the mixture is 1:500. Another Dick test is made at the same time to serve for purposes of comparison. A measure of the antibody content will give valuable data in showing the suitability of donors of blood serum for prophylaxis and for treatment. Normal or "naturally" immune donors can thus become available for this purpose. Another very important result of our ability to measure the antibody content of a serum is that it will enable us to determine one of the important characteristics of extracellular toxins, which is so well recognized in the case of diphtheria toxin, *i. e.*, the property of a toxin to be neutralized in multiple proportions by an antitoxic serum. Such studies are being conducted at the present time.

We have applied the Dick test to nearly one hundred cases of scarlet fever during the first four days of the disease and found them to be positive in all but three. As the patients progress into convalescence they nearly always show a negative or a pseudo reaction.

Two of the patients tested gave doubtful and one a negative reaction. The blood serum of the latter patient, taken on the fifth day of illness, showed strong blanching of a scarlet rash. The patient's history also indicates great doubt as to the diagnosis of scarlet fever. He had a marked rash, frequently described by the term "boiled lobster rash", but at no time did he have a sore throat. The fauces and tonsils were not congested. The rash began with a marked general pruritus. There was a heavy desquemation, which did not begin on the hands and soles of the feet, but in large flakes all around the neck. It gradually extended over the rest of the body.

There are four different reactions that can be distinguished with the Dick test and these reactions correspond closely to the similar four reactions that are noted with the Schick test. There is a positive, a negative, a pseudo and a combined reaction.

The positive reaction closely resembles at the end of 24 hours a positive Schick test which has reached its maximum on the third or fourth day. It appears within 6 to 12 hours, reaches its height at the end of 24 hours, and fades fairly rapidly; a good positive reaction is followed by pigmentation, with very slight or no scaling. The pigmentation may persist for a week or longer.

The pseudo reactions are due to the autolyzed bacterial substance of the streptococcus and other proteins in the test fluid. These reactions are not specific, as individuals who show a pseudo reaction will show similar reactions with the autolyzed protein of streptococci from different sources, such as abscesses, etc. To separate the pseudo reactions from the positive reactions I am using as a control a test toxin neutralized with an equal amount of 50 per cent convalescent serum or of normal serum from negative Dick reactors. In individuals giving pseudo reactions the local effect of the test fluid is not neutralized by the convalescent serum, the reactions on the two forearms, in the test and in the control, being of the same appearance. The pseudo reactions were seen in older children and especially in adults. They were also noted in some of those who gave a previous history of scarlet fever. *The results of the test become very confusing unless a control is made; then the results can be interpreted with little difficulty.* The combined reaction shows a much more pronounced redness at the site of the Dick test as compared with the control test. The positive and combined reactors are probably susceptible, the negative and pseudo reactors are probably immune to scarlet fever. The test is being applied now in institutions and homes where scarlet fever has developed and the actual experience will soon prove whether the clinical test and the corresponding serum findings (blanching of a scarlet fever rash and neutralization of the test toxin) can be depended upon.

The presence of varying amounts of antitoxic bodies in the serum of different individuals accounts probably for variations in the intensity of the clinical symptoms seen in cases of scarlet fever.

The toxin has been studied as to its ability to resist destruction by heat. At 100° C. for 45 minutes the toxin is destroyed in dilutions of 1:100. The pseudo-action of the protein is not interfered with by the heat. We will probably use the heated toxin for purposes of control, just as we have recommended the use of heated diphtheria toxin as a control in the Schick test.

The toxin does not seem to have as intense a local effect as the diphtheria toxin. The local reactions, however, seem to be in proportion to the dilutions of the toxin when compared with an arbitrary standard. Such a standard will have to be more definitely fixed before long to enable the work to be carried out everywhere in a more uniform manner.

The guinea pig showed only a slight effect and the mouse practically no effect to the intradermal or subcutaneous injection of the undiluted toxin. The rabbit reacts with rapidly diminishing intensity and shows only a slight reaction to a dilution of 1:100. Standardization on this animal may be subsequently worked out in a more satisfactory manner. At the present time the standardization of a toxin is most satisfactorily carried out by testing it intradermally in different dilutions in susceptible individuals.

The local effect of the toxin in the skin cannot be counteracted by a subsequent injection of an antitoxin containing serum. After a positive reaction has fully manifested itself at the end of 24 hours it cannot be blanched out by the injection of convalescent serum into the local reaction.

The positive reaction noted in all early cases of scarlet fever and the subsequent negative or pseudo reaction during convalescence in nearly all these patients would indicate that the different agglutinative strains of hemolytic streptococci found in the throats of scarlet fever patients produce the same antitoxic antibodies. Dr. Anna W. Williams and her co-workers in the Research Laboratory have found that only 35 per cent of strains could be put by their agglutination characteristics into one group. This is important as similar observations on the unity of toxin have been made in connection with different agglutinative strains of the bacilli of diphtheria and tetanus.

The Dick test has been found very valuable for diagnostic purposes, as a positive reaction obtained during the first day or two of a suspicious scarlet like rash and again two weeks or more after the fading of the rash will indicate that the patient did not have scarlet fever. The test is of great value and will find, like the Schick test, an increasing field of application in the selection of susceptible individuals for passive and active immunization; for passive immunization with convalescent serum from human beings or antitoxic serum from animals and for active immunization with increasing doses of the toxin itself. The negative retest would indicate the development of antitoxic immunity. Such studies in active immunization with diluted toxin are being carried out at the present time. I am using the following doses of toxin for active immunization, *i. e.*, 25, 50, 100 and 200 skin test doses, the injections being given intramuscularly about a week apart. The retest to determine the development of antitoxic immunity should be made after two to three months. The local reactions are only slight in positive reactors, but more marked in pseudo and combined reactors.

With the discovery of the toxin producing power of the hemolytic streptococcus a wide field of productive research has been thrown open to the investigator. Many problems will, no doubt, very soon be solved, such as the identity or lack of indentity of toxins produced by different strains of hemolytic streptococci obtained from a variety of sources. The subject of erysipelas and the possibility of producing an effective antitoxic serum will have to be reinvestigated. Eight years ago I used, with striking results, the convalescent whole blood from a case of erysipelas in the treatment of a young child, intensely ill with erysipelas.

The diagnosis of varying forms of sinus disease, such as ethmoid, sphenoid, antrum, ear infections, etc., will be facilitated as regards a doubtful scarlet fever etiology, if the strain of hemolytic streptococcus causing the local condition is obtained, a toxin produced and it is subsequently determined whether the toxin can be neutralized with convalescent scarlet fever serum.

154 (2386)

The influence of thyroid substances on the absorption of pleural effusions.

By C. S. DANZER.

[*From the Medical Service of Dr. Harlow Brooks, City Hospital, New York City.*]

A good many patients suffering with pneumonia develop during the course of their disease pleural effusions. Some of these effusions are small and assume no clinical importance since they are usually absorbed at or soon after the crisis. Others are large and manifest themselves by physical and röntgenological signs. They last for a number of weeks, are accompanied by moderate fever prolonged convalescence and sometimes undergo purulent metamorphosis. The gross appearance of the fluid is usually straw-colored, of varying transparency and usually bacteria-free on culture.

The pathogenesis of these effusions is wholly unknown. It is possible that a certain type of pneumonia produces pleural exudation. This, if true, might explain the great frequency of this complication in the so-called influenza pneumonias. It can by no means, however, be said that the parapneumonic pleural effusions are confined to this type of pneumonia. It occurs frequently enough in conjunction with fibrinous lobar pneumonia.

Perhaps certain individuals particularly predisposed develop effusions in the pleural space in the course of their pneumonias. This idea seemed not unlikely in the light of Eppinger's work on edema.

Eppinger[1] found that certain cases of cardiac or kidney disease were frequently typified by an enormous generalized subcutaneous edema, that treatment of the heart by digitalis, restriction of the fluid intake, a salt-free diet or the administration of diuretics had no effect. In such cases the feeding of thyroid gland produced a marked diuresis, the disappearance of edema and distinct clinical improvement sometimes for an extended period.

He subjected these clinical observations to experimental investigation and found that there was a striking difference between the absorption of subcutaneous fluid from a normal and thyroidectomized animals. While normal animals absorbed considerable amounts of saline from the skin, thyroprivic animals could not absorb saline so injected. In such animals a subcutaneous edema collection was produced. Also the effect of the drinking of water on diuresis differed in the normal and thryroidectomized animal. While diuresis was produced in the former, in the animal without a thyroid gland diuresis was insignificant or entirely absent.

These examples illustrate the governing influence of the thyroid secretion on the water transport from the subcutaneous tissues and also on diuresis.

Eppinger then argued, as did Hertoghe[2] before him, that there might be mild or *formes fruste* of hypothyroidism, just as there are of hyperthyroidism, that these forms of hypothyroidism might remain latent until an edema factor—heart or kidney insufficiency—was super-added. Now the edema would be out of all natural proportions and clinical improvement would follow only when thyroid was administered.

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[1] Eppinger, H., *Zur Pathologie u. Therapie des Menschlichen Odems.* Springer, 1917.

[2] Hertoghe, M., *Rolle der Schilddrüse bei Stillstand u. Hemmung des Wachstums,* J. F. Lehman, 1900.

It occurred to us that a similar explanation might be given for the parapneumonic effusions just discussed. This seemed plausible in the light of another known clinical fact. A tissue inflammation (furuncle or a cellulitis) in a patient suffering from circulatory failure with edema will be enormously swollen and doughy. Then, by analogy, a pneumonia (an inflammation of the lung) in an individual with a tendency to edema (latent or *formes fruste* hypothyroidism) may produce an extensive pleural edema or effusion. With this theoretical background we applied thyroid substances in four cases of pleural effusion, three of which were associated with pneumonia; the fourth one was primary and probably due to latent pulmonary tuberculosis.

Out of the three cases of parapneumonic pleural effusions thyroxin was used in two cases, dessicated thyroid gland in one, and in the case of tuberculous pleural effusions both of these thyroid substances were used.

Case 1.—A thirty-five year old man with a parapneumonic pleural effusion of one week's standing received 1/6 mg. of thyroxin every four hours for three days and 1/3 mg. every four hours for the next four days. After the three day period there was a striking diminution in the amount of fluid and after seven days the fluid was gone. This was demonstrated both by physical and x-ray examination.

Case 2.—Forty-six year old man with parapneumonic pleural effusion on the right side, of three weeks duration with no apparent tendency to spontaneous absorption, showed a marked diminution in the signs of fluid after three days of thryroxin, 1/6 mg. three times a day, and almost a complete disappearance after the next four days of thyroxin medication given 1/3 mg. every four hours.

Case 3.—Sixty year old woman with a parapneumonic effusion of two and one-half weeks duration, with no demonstrable tendency to spontaneous absorption, was put on dessicated thyroid gland three grains per day for four days. Physical and x-ray examination done on the fourth day of thyroid therapy showed that the fluid had been absorbed.

Case 4.—A thirty-seven year old woman with a primary pleural effusion, probably tubercular, was given dessicated thyroid gland eight grains per day for three days, with no evidence of diminution in the amount of fluid in the chest. Thyroxin, 1/6 mg., was given every three hours for the next three days with no change. She was again put on dessicated thyroid gland for four days more and now there was just a slight diminution in the pleural exudate. This difference, however, was probably spontaneous and not due to the thyroid therapy.

SUMMARY

Three cases of pleural effusion were treated with thyroid substances and showed a distinct tendency to rapid absorption. These were the effusions accompanying pneumonia, the so-called parapneumonic effusions. One case of primary pleural effusion, probably tubercular in nature, was not affected by thyroid administration.

Further observations will be necessary to determine which type of effusion in the pleura may be influenced by thyroid administration, also whether it is the individual (hypothyroid?) or particular type of effusion etiologically, that can be affected by thyroid administration.

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155 (2387)

On the coagulation of milk by rennin.

By ERNEST F. BOSTROM.

[*From the Department of Physiology, University and Bellevue Hospital Medical College, New York City.*]

The effects of various salts on the coagulation time of milk by rennin have been studied. It was found that $CaCl_2$, $MgSO_4$, and NaCl in certain dilute solutions favor coagulation (shortening the coagulation time), in other more concentrated solutions counteract it.

The coagulation of milk by rennin is sometimes spoken of as a precipitation of the hypothetical caseinogen by calcium salts. That it is not a true precipitation reaction is shown by the above mentioned action of salts. These to the same extent as they favor the stability of the milk colloids also favor coagulation, and vice versa; in other words, to the extent salts increase or decrease the surface charges of the colloids they act as coagulants or anti-coagulants. The effect of salts on the surface charge is expressed by the well-known equation:

$$\text{P. D.} = \frac{58}{2} \log \left(1 + \frac{z}{y} \right)$$

where z stands for ions in combination with the colloids, y for the same ions in the medium.

A condition under which an increase in the surface charges would favor the cementing together of the colloids may occur if colloids with the opposite surface charge make their appearance, in this case colloids which in Loeb's terminology can be written $R\,{}^{NH_2}_{COOH}$. Some calcium compounds are of that nature because they have their isoelectric point far toward the alkaline side. Calcium caseinate has, according to Loeb, its isoelectric point at pH 10.53.

If such a calcium compound plays a rôle in the clotting of milk by rennin it follows that clotting should not take place if the milk is more alkaline than 10.53. This test applies only to the last stage of clotting. In order to let the first stage take place undisturbed by alkalies and to prevent the last stage from occurring until the desired alkalinity had been obtained, the following procedure was adopted: the rennin was added to the milk after the calcium of the milk had been precipitated by oxalate (Morgenroth's method). After allowing about 5 minutes for the enzyme action, the H ion concentration of the milk was brought near the desired value and calcium added. It was found in this way that the last stage of clotting would not occur unless the milk was more acid than about pH 10.

The first stage of clotting, *i. e.*, the enzyme activity, does not take place at a pH more alkaline than 6.9. This is not due to a destruction of the enzyme by alkalies, because it was found that in the concentration used in these experiments rennin could be kept at a pH of 8.2 one hour and still, if neutralized, cause coagulation.

156 (2388)

Anisocoria after bilateral sympathicotomy.

By JOSEPH BYRNE and CARL P. SHERWIN.

[*From the Research Laboratory, Fordham University, New York City.*]

The fundamental mechanistic factors in the Schafer phenomenon consisted of pseudo- and true paradoxical phenomena induced in part by the sympathetic section and in part by lesion of the nociceptive paths at the first operation. The mechanistic factor in the second operation hinges upon the "shock" incidental to the general surgical procedure rather than the second sympathetic section. The Schafer phenomenon can be duplicated by operations in other parts of the body, *e. g.,* by section of one sciatic followed some days later by the section of the other sciatic. The most interesting feature of the Schafer phenomenon is the shortening of the period of incubation for true paradoxical dilatation phenomena. This is induced by the shock of the second operation which causes suspension of function in the decentralized neurones situated within, and distal to, the superior cerivcal ganglion.

157 (2389)

Studies on purification of antibodies. II.

By R. OTTENBERG and F. STENBUCK.

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This paper is the continuation of one delivered before this Society[1] describing the metal precipitation and purification of typhoid agglutinating extract. The extract is prepared by sensitizing typhoid bacilli with antityphoid horse serum, washing the complex with saline to remove horse serum and subsequently dissociating agglutinin from bacteria by means of weak alkali.

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[1] Ottenberg, R., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 14.

In that paper it was stated that a M/200 final concentration of $CuCl_2$ was used to precipitate agglutinin from the extract. We have found that at the proper hydrogen ion concentration, much smaller amounts of copper will precipitate all the agglutinin. The limiting concentration of copper seems to vary with different extracts, and with the same extract at varying hydrogen ion concentrations. For instance, an extract agglutinating in a dilution of 1:640 was precipitated with copper chloride. When 1.0 cc. N/40 HCl was added to 10 cc. of extract before the addition of copper, the final concentration of M/4400 $CuCl_2$ was sufficient to bring down all of the agglutinin in the precipitate, while final concentrations of M/6600 and M/8800 brought down half the agglutinin and a final concentration of M/17600 did not bring down any.

When only 0.75 cc. N/40 HCl was added to 10 cc. of extract before addition of copper chloride, a final concentration of M/4400 brought down no agglutinin in the small amount of precipitate that formed and a final concentration of M/2500 of copper chloride was necessary to recover all the agglutinin.

In purifying the extracts by copper precipitation an orienting experiment is first performed. Portions of 10 cc. of extract are placed in centrifuge tubes and varying quantities of N/10 HCl added. To each tube is added 1 cc. of M/400 copper chloride solution and water to bring the total volume to 12 cc. Within certain limits of acidity a cloudy precipitate forms in the tubes. The tubes are allowed to stand in the icebox for 24 hours, when the precipitate has generally flocculated and fallen to the bottom and sloping sides of the tube. The tubes are centrifuged to pack the precipitate and the supernatant liquid is then poured off. At each end of the range of precipitation the liquid does not become entirely clear even on repeated centrifuging.

The precipitate is then dissolved in a volume of N/200 HCl equal to the original volume of extract taken. The pH of the supernatant is determined and both supernatant and dissolved precipitate examined for agglutinating power (24 hour incubation, room temperature).

It is found that the tubes at the end of the series do not have all of the agglutinin in the precipitate, but some is demonstrable in the supernatant fluid. The supernatant fluids from the tubes in the middle of the series have some agglutinin present, generally agglutinating in a dilution of 1:40 and 1:80 and rarely as high as 1:160. The range of pH in the tubes showing precipitates is from about 4.6 to 7.2 with optimum precipitation at about 6.4 as has been found before. It is also noteworthy that in almost every instance the dissolved precipitate shows a definite increase in agglutinating value over the original extract, notwithstanding the fact that there is also some agglutinin recovered in the supernatant liquid. For example, the copper precipitate of an extract agglutinating in 1:800 dilution when dissolved in N/200 HCl was found to agglutinate at 1:900 and sometimes 1:1000, and in addition the supernatant fluid agglutinated at 1:40 or 1:80. This phenomenon may be due to either or both of two causes, (1) the removal of extraneous bacterial matter which interferes with agglutination by combining with agglutinin, or (2) the influence of copper on agglutination (note—copper chloride alone in M/500 solution does not agglutinate). We have excluded the possibility of acid agglutination by hydrogen ion determination of the test mixtures; the buffer effect of the bouillon as well as the great dilution of the acid alone are enough to explain this.

Using the tube showing most agglutinin in the precipitate with least volume of precipitate as a guide to optimal proportions, a large quantity of the extract is precipitated by adding a corresponding amount of acid and copper chloride solution. The mixture is allowed to stand in the icebox 24 hours and then centrifuged to separate the flocculent precipitate. The precipitate is then dissolved in a volume of N/200 HCl equal to the volume of extract used. The supernatant and dissolved precipitate are analyzed chemically and examined for agglutinating power.

Ten cubic centimeter portions of the dissolved precipitate are then treated in the same manner as the original extract with the difference that the addition of copper chloride is now omitted.

In this second precipitation there is a slight narrowing of the reaction zone, precipitation occurring between about pH 5.0 and 7.0 with a maximum again at about pH 6.4.

A large volume of the dissolved first precipitate is now treated with acid to bring it to the desired pH, allowed to stand 24 hours in the icebox and centrifuged. This second precipitate is then treated in the same manner as the extract and first precipitate and a third precipitate obtained, which is in turn dissolved in N/200 HCl.

By following the above technique we have been able to prepare a solution of typhoid agglutinin which we believe to be purer than any hitherto prepared. The dissolved third precipitate agglutinated completely in 1:900 dilution, with partial agglutination up to 1:1200. It had a nitrogen content of .00042 gm. per 100 cc. (determined by the Kjeldahl method on 100 cc. sample). The agglutinating power was diminished about 22 times, while the nitrogen content was diminished 3450 times. Thus the nitrogen was diminished 156 times as much as the agglutinin. The dissolved third precipitate, when examined by the usual tests gave the following results:

	Original serum	Serum 1/3500[1]	Original extract	Third precipitate
Titre	*++ 12800 + 25600	++ 4 + 8	++ 800 + 1100	++ 900 + 1200
Nitrogen	1.45 gm. per 100 cc.	0.00041 gm. 100 cc. calculated	0.0031 100 cc.	0.00042 gm. 100 cc.
Millon	+	+	+	—
Ninhydrin	+	—	—	—
Biuret	+	+	+	
Xanthoprotein	+	+	+	?
Kuttner reaction[2]	+	+	+	—
Adamkiewicz	+	—	—	—
Sulfur		—	—	—
Phosphorus				trace

* ++ indicates complete and + incomplete agglutination.

The dissolved third precipitate and the original extract were tested for specificity. They did not agglutinate *B. paratyphoid* A or B, *B. dysenteriæ* (Flexner and Shiga types), *B. coli communis* in dilutions of 1:10 or higher. Copper chloride alone agglutinated in M/330 concentration but not in M/500.

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[1] This dilution was chosen because it represents the same nitrogen content as the third precipitate.

[2] Kuttner, T., PROC. SOC. EXP. BIOL. AND MED., 1918, xv, 91. This is a color reaction for NH_3 and free amino groups.

The amount of copper added to the extract in the first precipitation was 0.01816 gm. per liter. The first supernatant fluid had a copper content of 0.0146 gm. per liter. Thus the precipitate had in it 0.00356 gm., representing one liter of extract. Fifty cubic centimeters of the dissolved third precipitate showed no detectable copper when the organic matter was destroyed by nitric acid, the material evaporated to dryness, taken up in concentrated hydrochloric and again evaporated to dryness, taken up in very dilute hydrochloric acid and examined by the ferrocyanide method.

The above tests would seem to preclude agglutinin being a protein, or even a protein hydrolysis product. The small quantity of nitrogen may be in agglutinin, or may be due to a small amount of nucleic acid present as an impurity from the bacteria.

Since it is known that the method of extraction, namely weak alkali, will extract nucleic acid from bacteria, and since the nucleic acids are precipitable by copper, the nitrogen present may well be in the form of nitrogenous base as an impurity derived from the bacteria. If so, possibly the agglutinin is represented by carbohydrate. On the other hand, the carbohydrate itself may also be derived from the bacterial nucleic acid. In this case the chemical factors representing the agglutinin may be so minute in amount as to escape detection entirely. Analyses now in progress may throw some light on this question.

158 (2390)

The isolation of a crystalline substance (M. P. 223°C) having the properties of "bios."

(Preliminary report).

By WALTER H. EDDY. RALPH W. KERR and R. R. WILLIAMS.

[*From the Laboratory of Physiological Chemistry, Teachers College, Columbia University, New York City.*]

A crystalline substance melting sharply at 223° C. has been isolated by the use of differential adsorbents from autolyzed yeast which in very small amounts (0.005 mg. per cc. of Ful-mer's medium F.) produces during a 24 hour incubation at 31° C. a volume of yeast growth fifteen to twenty times that in the controls.

Microphotographs made with the petrographic microscope were exhibited to demonstrate the homogeneity of the crystalline product together with evidence obtained by recrystallizing the product first from water and then twice from alcohol.

The recrystallized product retains its constant melting point and shows no loss of activity.

Curves based on yeast tests made with varying concentrations of autolyzed yeast and of the crystals were shown to demonstrate that the form of curve is identical in the two instances, both showing an optimum concentration and lesser stimulation for concentrations on either side of the optimum concentration.

The yield of the product was about 0.03 per cent of the dry weight of the autolyzed brewer's yeast used. The carbon and hydrogen percentages have been determined and are: Carbon, 43.29 per cent; hydrogen, 8.31 per cent. The nitrogen content has not yet been accurately determined but will apparently run in the neighborhood of 25 per cent. The substance gives no ninhydrin or biuret reaction. It is freely soluble in cold water, in acid and alkaline solutions and in dilute ethyl alcohol. In cold 95 per cent alcohol it is sparingly soluble but passes readily into solution when the alcohol is heated. It is only slightly soluble in 100 per cent acetone but very slight dilution of the acetone with water brings about its solution.

The essential feature of the method of isolation consists in a preliminary purification of autolyzed brewer's yeast with fullers earth which removes other substances than bios, and then the use of ferric hydroxide in colloidal sol which under specific conditions devised by Dr. Kerr removes the bios and can later be dissolved and the bios freed from cantaminating ions. Details of the process are still undergoing further refinement and will appear in a later publication.

The expense of this investigation was met in part by a grant to the University by the Fleischman Company, and we are also indebted to the Jacob Ruppert Company for the brewer's yeast used in the studies.

159 (2391)

Blood concentration in insulin hypoglycemia.

By D. L. DRABKIN, I. H. PAGE and D. J. EDWARDS.

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

Fisher and Wishart[1] found that, following the hyperglycemia due to the ingestion of large amounts of glucose, a temporary blood dilution and anuria resulted. Barbour[2] found that glucose exerted an antipyretic effect in dogs with peptone fever. In subsequent communications this observer, in collaboration with other workers[3], has shown that a hydremic plethora due to hyperglycemia is the basis of antipyretic action. From this work it would appear that, under certain conditions, an increase in blood sugar calls forth a mobilization of water into the blood.

Is the antithesis of such a condition, a dehydration of the blood in certain hypoglycemic states, possible? It seemed desirable to us to study from this standpoint the condition of insulin hypoglycemia.

The procedure was as follows:

1. The dogs were anesthetized with iso amyl ethyl barbituric acid.[4] A control experiment showed that *per se* this drug had very little effect either on blood sugar or on blood concentration.
2. Blood samples were withdrawn, in some experiments from the external jugular vein, in others from the femoral, and in still another from the vena cava just above the renal veins.
3. After drawing the "control" samples a large dose of insulin (20 units per kilogram body weight) was administered intravenously.
4. Blood samples were taken at suitable intervals following the insulin.

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[1] Fisher, G., and Wishart, M. B., *J. Biol. Chem.*, 1912-13, xiii, 49.

[2] Barbour, H. G., PROC. SOC. EXP. BIOL. AND MED., 1919, xvi, 136.

[3] Barbour, H. G., and Herrmann, J. B., *J. Pharmacol. and Exp. Therap.*, 1921, xviii, 165; Barbour, H. G., and Howard, A. J., PROC. SOC. EXP. BIOL. AND MED., 1920, xvii, 150.

[4] Page, I. H., *J. Lab. and Clin. Med.*, 1923, ix, 2.

5. Each blood sample was analyzed for hemoglobin, which was taken as the index of blood concentration, and for sugar. Hemoglobin was determined by the method of Cohen and Smith;[5] the blood sugar by the method of Shaffer and Hartmann.[6] A record of mean blood pressure was taken at frequent intervals during the experiment.

The experimental results may be summarized as follows:

1. Venous blood, following the administration of large doses of insulin, shows that, with a falling blood sugar percentage, there is an increase in concentration ranging in our work from 15.1 per cent to as high as 44.8 per cent.

2. A graphical record of a typical experiment shows that the blood sugar falls steeply during the first hour and a half after insulin, then more gradually. The percentage of hemoglobin rises simultaneously with the steepest gradient of the curve shown during the second hour. This record seems to indicate that first there is a fall in the blood sugar, then, after a short delay, blood concentration takes place. The mean blood pressure tracing shows a slight decline during the first two hours after insulin, then a tendency toward a gradual return to normal. This tracing shows the absence of circulatory failure. In the shock of circulatory failure there is extreme, sudden fall in blood pressure and, according to Gasser and coworkers,[7] blood concentration is the most constant finding in that condition. In our experiments hemoglobin and mean blood pressure tracings show a striking similarity of contour in opposite directions.

3. In some of the experiments in which the fatal issue came rapidly the fall in blood sugar and in concentration of the blood also came on rapidly. Throughout the course of the experiments certain related factors of blood concentration—apparent increase in viscosity, tendency to form clots—factors observed by Macleod[8] in some of his studies, were often noted and regularly appeared only in samples of blood with high hemoglobin and low sugar.

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[5] Cohen, B., and Smith, A. H., *J. Biol. Chem.*, 1919, xxxix, 489.

[6] Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1920-21, xlv, 365.

[7] Gasser, H. S., Erlanger, J., and Meek, W. J., *Am. J. Physiol.*, 1919-20, l, 31.

[8] Macleod, J. J. R., *Physiol. Reviews*, 1924, iv, 21.

4. In those experiments in which the urine volume has been followed, marked diuresis was observed, the rise in urine output apparently being directly related to the concentration of the blood and to the fall in blood sugar.

CONCLUSION

In insulin hypoglycemia a concentration of the blood is constantly found.

160 (2392)

Salt effects in bacterial growth: IV. The physical nature of bacterial growth in various concentrations of neutral salts.

By GEORGE E. HOLM and J. M. SHERMAN.

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The physical nature of growth produced by various organisms has generally been attributed to the characteristics of the organisms themselves. Those organisms producing a pellicle upon the media in which they are growing were thought to do so because they were more strongly aerobic than those which did not form pellicles.

Of recent years, however, this view has been changed and it is known that organisms which grow in the surface of the media or settle to the bottom as they grow, do so, not alone because of the characteristic of the organism, but largely because of the physical properties of the media. It has been shown by Larson, Cantwell and Hartzell[1] that the nature of the growth of *B. subtilis, Mycobact. tuberculosis* and other organisms can be entirely changed by the addition of castor oil soap. In the case of *B. subtilis* 45 dynes prevents the formation of pellicles and growth takes place down in the body of the medium.

Neutral salts when added to the media in small quantities affect the physical nature of the growth of various organisms. *Bact. coli* when grown in medium of 1 per cent peptone broth whose H ion concentration has been adjusted between pH 5 and pH 8, shows great and varying degrees of turbidity, but the majority of the organisms settle out spontaneously. It has been noticed in our work, however, that when a small quantity of a neutral salt is added the majority of the bacteria are held in suspension and a very diffused growth is obtained.

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[1] Larson, W. P., Cantwell, W. F., and Hartzell, T. B., *J. Inf. Dis.*, 1919, xxv, 41.

Observations have been made upon media of varying H ion concentration in order to determine whether or not the action of the neutral salts is primary or secondary and to determine the relation of turbidity to the rate of growth in various concentrations of neutral salts.

EXPERIMENTAL

One per cent peptone was prepared and to various portions was added NaCl in quantities to make concs. of 0.05 M, 0.20 M, 0.30 M and 0.40 M. These media were then adjusted to H ion concs. of pH 5.3, 6.3, 7.0, 7.7 and 8.3, sterilized and inoculated with *Bact. coli.* Table I shows the results of these experiments.

TABLE I.

The Effect of H ion Concentration and Various Salt Concentrations of NaCl upon the Turbidity and Growth of *Bact. coli.*

pH of media	Order of turbidity of various NaCl concentrations					
5.3	0.05	0.10	C.	0.20	0.30	0.40
6.4	0.10	0.05	0.20	C.	0.30	0.40
7.1	0.10	0.05	C.	0.20	0.30	0.40
7.8	0.10	0.05	0.20	C.	0.30	0.40
8.3	0.20	0.10	0.05	C.	0.30	0.40

In the 1 per cent peptone controls the order of turbidity with regard to the pH of the media was as follows: 7.8, >7.1, >8.5, >6.4, >5.4. In our former experiments we have found that this is the order for various freshly prepared peptone media when placed in the order for optimum growth.

With the addition of increasing amounts of NaCl there seems to be a gradual shifting of the conditions for maximum turbidity in the pH range over which the experiments were carried out. The region of maximum turbidity shifts slightly toward the more alkaline side. The difference here is very small between the various H ion concentrations, as far as turbidity is concerned, and from the table given in the second paper of this series it was noted that the difference in the rate of growth in this region and these concentrations of NaCl is very small. In other words, the conditions for maximum turbidity or diffused growth seem to parallel very closely the optimum conditions for rate of growth.[2]

A series of sodium salts with various anions were compared in order to obtain their relative effects upon the nature of the growth. NaI, $NaNO_3$, Na_2SO_4, and Na acetate were tried in concentrations ranging from 0.05 M to 0.40 M.

TABLE II.

The Effect of Various Concentration of Different Sodium Salts upon the Physical Nature of Growth of *Bact. coli* at pH 7.6.

Salts used.	Order of turbidity of the various salt concentrations.					
$NaNO_3$	0.10	0.20	0.30	0.40	0.05	C.
NaI	0.10	0.20	0.30	0.05	C.	
Na_2SO_4	0.10	0.05	0.20	C.	0.30	0.40
Na acetate	0.10	0.20	0.30	0.40	0.05	C.

Sodium iodide and sodium nitrate both give growths of a more highly dispersed character than the growth in the controls, even in concentrations of 0.30 and 0.40 M. Sodium sulfate, on the other hand, shows more dispersed growth than the control only in concentrations of 0.05, 0.10, and 0.20 M. On the other hand, 0.30 and 0.40 M sodium sulfate peptone solution give granular growths, showing the action of the SO_4 ion. Na acetate gives approximately the same results as NaCl.

When 0.20 M concentrations of these salts are compared with the same concentration of NaCl the order of the turbidities was found to be as follows: $NaNO_3$, >NaI, >NaCl, >control. This order again parallels very closely, as far as can be judged under the conditions used, the order found for the rate of growth. Upon allowing these cultures to stand for a time after they have been incubated for from 6 to 8 hours it was noted that the tendency for settling out was but slight in the media which showed maximum turbidity. From analogy with the effect of neutral salts upon colloidal properties in general, the dispersed growth which has been explained might be due to the initial lowering of the surface tension by small quantities of a neutral salt added to 1 per cent peptone. The magnitude of change of surface tension of 1 per cent peptone with added amounts of salts was measured.

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[2] Holm, G. E., and Sherman, J. M., *J. Bact.*, 1921, vii, 511; Sherman, J. M., and Holm, G. E., *J. Bact.*, 1922, vii, 465.

TABLE III.

Changes in the Surface Tension of Peptone upon Addition of Neutral Salts.

Media	pH	Surface tension (dynes/cm²).
1 per cent peptone	5.0	58.03
“ — .20 M NaCl	5.0	57.95
“ — .10 M Na_2SO_4	5.0	57.68
“ — .20 M Na_2SO_4	5.0	57.30

Table III indicates that there is but a slight decrease in the surface tension of 1 per cent peptone when small amounts of NaCl or Na_2SO_4 are added. The changes are so small, however, that it would be impossible to attribute the cause of dispersed growth to the changes in the surface tension. Furthermore, it was found that 0.20 M Na_2SO_4—1 per cent peptone media. It is hardly probable, therefore, that the phenomena of dispersed growth obtained when small amounts of neutral salts are added is due entirely to changes in surface tension.

Hofmeister[3], and Fischer[4] showed that small amounts of neutral salts increase the swelling of proteins. The Cl, NO_3, and I ions were found to be the most efficient in this respect. Hofmeister and Bechhold and Ziegler[5] showed that diffusion through membranes may be increased by the presence of chlorides, nitrates, iodides, etc. The permeability of plant plasma for neutral salts has been studied by Kahho,[6] who found that iodides, bromides, chlorides, etc., favored permeability more strongly than the sulfate and citrate. He also noted that all ions which increased permeability also increased susceptibility to toxic action. It has been shown by Lemon[7] that bacteria are less resistant to the action of phenol in the presence of small neutral salt concentrates than in the ordinary media in which they are in suspension. The phenomenon is in all probability due to the increased permeability of the bacterial cell.

Entirely in accord with these findings we may conclude that in the presence of small amounts of neutral salts the permeability of the cells is greater than the permeability of the same bacterial cells in the plain 1 per cent peptone solution. This may mean more highly hydrated and larger cells, which would tend toward greater stability of the suspension. This is in accord with the phenomena observed by Henrici,[8] through microscopic measurements of the size of *B. megatherium* during the lag period and during the period of most rapid growth. It was found that during the period of most rapid growth the organisms are larger in size than during the lag period or during the period subsequent to the period of rapid growth.

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[3] Hofmeister, F., *Colloids in Biology and Medicine*, p. 346.

[4] Fischer, M. H., *Colloids in Biology and Medicine*, p. 80.

[5] Bechhold, H., and Zeigler, J., *Colloids in Biology and Medicine*. pp. 55, 346.

[6] Kahho, H., *Biochem. Z.*, 1921, cxxiii, 284.

[7] Lemon, J. S., *J. Phys. Chem.*, 1920, xxiv, 570.

We have shown in previous work that small amounts of neutral salts increase the rate of growth in 1 per cent peptone and we believe that this increase in the rate of growth is closely related to the permeability of the bacterial cell. Largely because of the observation that there is a remarkably close correspondence between optimum salt concentration for maximum turbidity or diffused growth, and the optimum salt concentration for the maximum rate of growth we are inclined to believe the effect of the salts, whatever it may be, is in this case an effect upon the organisms themselves rather than an effect upon the physical property of the media.

161 (2393)

On the factors influencing the appearance of placques of bacterial lysis.

By J. BRONFENBRENNER and CHARLES KORB.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

If the activity of "bacteriophage" is studied upon agar cultures spots or so-called placques of lysis are seen to appear, instead of a homogeneous growth. As a rule, these clear spots stand out quite distinctly against the background of agar, but occasionally one observes among them a certain number of spots which may be considerably less distinct than others. The variations among the spots are even more marked as regards their size. The placques may vary in the same plate from the size of a pin-prick to that of a size larger than the head of the pin. A number of authors have succeeded in demonstrating that by isolating and propagating each of the variants one can obtain pure strains of "bacteriophage"—showing very little if any variation in the size or distinctness of the placques (or spots). In their opinion the co-existence in one plate of spots of different size is indicative of impurity of the lytic strain.

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[8] Henrici, A. T., Proc. Exp. Biol. and Med., 1924, xxi, 215.

Having in our possession a strain of "bacteriophage" which showed a tendency to give rise to spots of lysis of various sizes, we considered it impure and attempted to isolate the several component strains by the usual method of daily transfers. When we found that pure strains of "bacteriophage" thus secured would unexpectedly show marked changes in respect to the average size of spots or their distinctness, we attempted to ascertain the cause of the changes. With this end in view we made a systematic study of the factors involved in the placque formation and made the following observations.

1. Within certain limits, the average size of the placque changes inversely with the number of bacteria susceptible to lysis present in each cc. of agar.

2. The average size of the placque depends upon the relative concentration of young and old individuals in the culture—the greater the number of old bacteria, the smaller the spots.

3. While the ratio between the number of resistant (capable of multiplying in the presence of bacteriophage) and susceptible bacteria has no marked effect upon the size of the spots, this ratio influences their distinctness. The greater the relative number of resistant bacteria, the less distinct are the spots. The difference in appearance between the two types of spots is particularly evident when observed under the low-power of the microscope, the less distinct spots often being indistinguishable from the surrounding growth.

4. The concentration of agar has a most marked effect upon the size of the spots. When the agar is more concentrated the spots are smaller.

Since we observed in another connection that the hydrogen ion concentration and the concentration of salt in the medium may affect the appearance of the plaques, we wish to state that in these experiments the pH of the medium was 7.4 and the only salt present was 0.4 per cent sodium phosphate.

162 (2394)

Ionic nature of botulinus toxin.

By J. BRONFENBRENNER.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

In a recent paper Northrop[1] suggested a method which makes it possible to inquire into the ionic nature of such substances as cannot be obtained in a state of chemical purity. The results obtained by Northrop in the study of the behavior of trypsin agreed with the prediction which could be made on the basis of the Donnan equilibrium theory if trypsin is considered as a positive monovalent ion in the zone of hydrogen ion concentration at which the tests were performed.

In view of the peculiar behavior of botulinus toxin under the influence of various conditions, and particularly of changes in hydrogen ion concentration, we suspected for some time that this toxin may be ionic in nature. The method of Northrop offered the means for ascertaining such a possibility.

It was found that toxin is distributed between the gelatin and the fluid in approximately the same ratio as chlorine ion, and inversely as hydrogen ion. This suggests that botulinus toxin is a monovalent, negatively charged ion, at least between pH 2 and pH 8. It was found that at the hydrogen ion concentration of iso-electric gelatin the ratio of distribution of toxin between the gelatin and fluid was about 1; at pH 2 it was about 3; at pH 7.8 it was about 0.5. Excess of salts depressed the ratio to 1 in all instances.

Incidentally it was found that at the hydrogen ion concentration of iso-electric gelatin, about 60 per cent of toxin was lost. At the pH of 7.8 about 90 per cent of toxin was lost. At pH 2 to 3, the potency of the toxin increased over 100 per cent.

I wish to thank Doctor Northrop for his helpful suggestions.

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[1] Northrop, J. H., *J. Gen. Physiol.*, 1924, vi, 337.

163 (2395)

Further studies on the potency of botulinus toxin.

By J. BRONFENBRENNER.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

Repeating titration of toxin from day to day, we have observed that determination of the potency of dilutions of toxin in physiological salt solution has often given inconsistent results. Inquiry into the cause of this inconsistency brought out the fact that NaCl, as well as many other salts of mono- and polyvalent metals, have direct deteriorating effect upon toxin. This effect is more marked the higher the dilution at which the toxin is exposed to the salt. At any given concentration of toxin in a salt solution, deterioration is greater the longer the exposure and the higher the concentration of salts.[1] An attempt to neutralize the effect of a single salt by balancing with other salts having antagonistic physiological action yielded no definite result. If, instead of using salt solution, the toxin is diluted in distilled water, the results of titration become more regular, and solutions of toxin keep fairly well. On the other hand, in the presence of a low concentration of salts, addition of serum or broth seems to protect the toxin from deterioration. Moreover, if the toxin is diluted not in salt solution or distilled water but directly in normal horse serum or even in ordinary broth, its potency is increased as compared with that of the same toxin diluted in distilled water.

In view of the tendency of foreign serum to increase the toxic action of botulinus toxin, it is particularly important to determine carefully the type of toxin involved before administering antitoxin. Actual experiments have shown that at least the animals receiving heterologous antitoxin (type B) together with botulinus toxin (type A) die more promptly than the controls receiving the toxin alone.

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[1] We are at present immunizing animals with such detoxicated filtrates of cultures of *Bacillus botulinus.*

164 (2396)

Operative results of thyroidectomy and thymectomy in the rabbit.

By LOUISE PEARCE and C. M. VAN ALLEN.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

During the last two years, our study of animal resistance has included investigations on the endocrine mechanism as one of the factors involved in the reaction of the host to disease processes.[1, 2, 3] Operative procedures of various types have been carried out on the thyroid, thymus, and suprarenal glands of rabbits, and the majority of animals have been inoculated with *Treponema pallidum* or a malignant tumor of the rabbit[4] either before or after operation. The rabbits so operated upon have been kept under observation from three to ten months, and at the conclusion of the experiments a complete autopsy was carried out in each instance. In this communication we wish to present a summary of the operative results upon the thyroid and thymus from the point of view of operative mortality, wound infection, and the incidence of residual nodules of gland tissue.

Complete thyroidectomy has been performed in 103 instances with an operative mortality of 3.9 per cent (4 rabbits). These deaths occurred among our early operations and were attributed to over-anesthetization; in one instance there was an acute pulmonary emphysema. We have had no fatalities in the last 54 operations. The only case of wound infection occurred in the one animal in which local anesthesia was used and in which there was also an extensive mange. Up to the present time, 90 of the

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[1] Brown, W. H., and Pearce, L., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 472.

[2] Brown, W. H., and Pearce, L., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 476.

[3] Pearce, L., and Van Allen, C. M., *Trans. Assoc. Am. Phys.*, 1923, xxxviii, 315.

[4] A description of the original tumor, its successful transplantation, and the studies carried out on subsequent generations will be found in recent issues of the *Journal of Experimental Medicine* beginning with: Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, xxxvii, 601.

rabbits have been autopsied, and in four instances (4.4 per cent) tiny nodules of hyperplastic thyroid tissue have been found in close connection with a silk ligature.

A partial removal of the thyroid has been carried out in 30 rabbits. In 24 instances one lobe and the isthmus were removed while in six the operation included the ligation of the blood supply to the remaining lobe. There has been one operative death, or 3.3 per cent, and no instance of wound infection.

The thymus has been completely removed by a transthoracic operation from 50 rabbits with an operative mortality of 10 per cent. One death was due to air embolism, three to an acute pulmonary edema, and the last was probably due to the combination of an injury to the left phrenic nerve with an extensive right pneumothorax. There were 18 instances of unilateral pneumothorax; none of these animals died or appeared to be adversely affected by this complication. A local, well encapsulated, mild or moderate (two cases) infection in the operative region, either inside or outside the chest, occurred in eight rabbits, an incidence of 16 per cent. None of these animals died. Autopsy records of 44 rabbits are at present available—in one instance what appeared to be a few small nodules of thymic tissue were found.

In our last series of 29 thymectomized rabbits which comprised 15 adult, 6 young, and 8 old animals, there was only 1 death occurring in an old animal, or a mortality rate of 3.4 per cent. Older rabbits present more difficulties in this operation than do young or adult animals. The operation in young rabbits from three to four months of age is comparatively easy. Furthermore, in this group of 29 rabbits, our infection rate fell to 6.8 per cent —two rabbits, and in both the infection was of a mild type.

In our experience, rabbits subjected to complete or partial thyroidectomy or complete thymectomy will live for many months in excellent physical condition even with the imposition of the additional stress incident to inoculation with various agents. Complete thyroidectomized animals, however, are definitely more susceptible to spontaneous diseases, such as snuffles, than are normal animals. A similar condition of lessened resistance induced by complete removal of the thyroid, is strikingly shown by the more severe course of experimental syphilis and of a transplantable malignant tumor in such rabbits.[8]

165 (2397)

Further studies on the physiological action of non-specific antigens prepared from shattered hemo-proteins.

By CLYDE BROOKS, GEORGE T. PACK and HENRY GOODE.

[*From the Department of Physiology, School of Medicine, University of Alabama, University, Ala.*]

During the past decade, one of us (Brooks) has made extensive investigations of new methods for combating infections. In the course of this study, the following hypothesis was formulated:[1] In the blood and blood-forming organs are found the various chemical structures, cellular or otherwise, which are responsible for the production of the antibodies, formed in the immunity response to infection. Consequently the blood should be good material from which to isolate immunifacient antigen bodies.

Acting upon this theory, a protein was carefully prepared by shattering the fibrin of ox-blood with hydrochloric acid and pepsin. The residue was fractionated by precipitation with ammonium sulfate, retaining the lower secondary proteoses, which are the most active components. These proteoses, he found, were multiple in number and varied in nature, but their very complexity may explain their service in combating infections of different etiology.

A host of workers has searched for a protein which would be efficient therapeutically, and yet rid of the disagreeable concomitants of protein action; namely, fever, chills, sweating, etc. The above mentioned proteose seems to fill this requirement. Brooks and Stanton[2] noted the absence of the so-called "characteristic protein reaction" and at first, on this account, were inclined to doubt the efficacy of this shattered hemo-protein antigen, but this view was dispelled by the remarkable success of this protein in a large series of cases of acute and chronic arthritis. The general opinion heretofore is best expressed by quoting Petersen[3]:

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[1] Brooks, C., Non-specific protein antigens prepared from shattered hemoproteins, *Science*, 1919, xlix, 196.

[2] Brooks, C., and Stanton, F. M. Non-specific hemo-protein antigen for the treatment of arthritis, *N. Y. Med. J.*, 1919, cix, 452.

[3] Petersen, W. F., Protein therapy and non-specific resistance, New York, 1922.

"There is a probability that . . . the positive phase or mechanism of recovery after non-specific injections is a function, or at least very closely related to the degree of the negative phase or the intensification of the disease process that is clinically manifest in the reaction of the patient. Just as in local foci of disease a marked augmentation of the inflammation, both after specific vaccine injections or non-specific injections, is followed as a rule by clinical improvement, so in the general infections a relatively severe reaction is more frequently followed by an abortive recovery than when the reaction is very mild or absent."

But the experience of Brooks and Stanton indicates that the depressing phase of the process is not an essential feature of antigen action, as exemplified by their results with shattered hemoprotein. The interpretation of other investigators, that the "reaction" is causal in promoting recovery, is probably a misconception, based upon the complicating factor of concurrent "reactions" with the injections of the proteins previously used. In fact, it is quite conceivable that the "reaction" often seen in foreign protein therapy is due to the action of *a certain group of proteins*, and the beneficial action is due to *an entirely different group of proteins,* and therefore if the two groups are successfully separated we may have, on the one hand, *the group of toxic proteins* which gives a marked "reaction" but has no therapeutic value, and on the other hand, *the group of therapeutic proteins* which has no toxic action, but acts therapeutically.

This is not the only difference between this hemo-proteose and other proteoses. Whereas the latter, upon injection, induce a primary leucopenia of varying duration and intensity, and a subsequent secondary leucocytosis, Brooks[4] found that his hemo-proteose by its leucotonic action, stimulated leucopoiesis immediately upon injection and that after several hours a well marked leucocytosis was apparent, without the primary intervening leucopenia. This is evidently a desirable and valuable pharmacological action.

With dogs, guinea pigs and rabbits as the experimental objects, we have attempted to produce sensitization to subsequent hemoproteose injections, but upon repeated injections of the protein, employing varying doses and intervals, we find that the animals

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[4] Brooks, C., The effect of shattered hemo-protein on the colorless blood corpuscles, *Am. J. Physiol.*, 1919, xlix, 127.

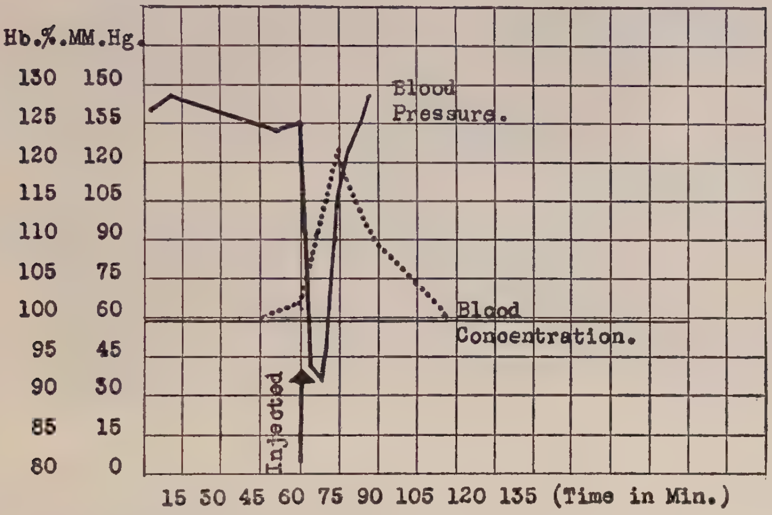

Chart One. Dog C. (0.2 gm. Brook's Hemoproteose injected intravenously per kgm. body weight of dog. Weight of dog 10.4 kgms.).

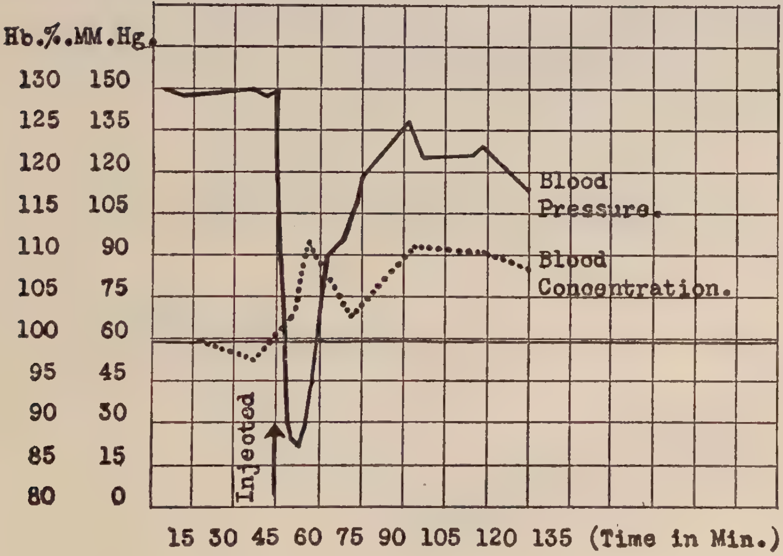

Chart Two. Dog B. (0.2 gm. Witte's Peptone injected intravenously per kgm. body weight of dog. Weight of dog 14.5 kgms.).

are not sensitized, but are more tolerant to successive doses. This apparent increased tolerance harmonizes with the desensitization noted clinically when the hemo-protein is employed. It also suggests that the hemo-protein could advantageously be administered in gradually increasing doses in certain cases. We are planning to test the possibility of sensitization in guinea pigs by intracranial injections.

How can the absence of these undesirable effects in the use of this new proteose be explained? Is it weaker than other proteins, and thus the dosage used too small to evoke a response? Experimental evidence is against this view. First, the therapeutic index is at least equal to that of other proteins. Second, the pharmacological action of this proteose is comparable to the action of other proteoses.

In the present paper we have attempted to compare the physiological activities of Witte's peptone with the action of this new hemo-proteose on blood concentration and blood pressure. Underhill and Ringer[5] have demonstrated that in dogs an increased blood concentration accompanies the shock induced by either Witte's peptone or by deuteroproteoses prepared from egg white. Our proteose, prepared from blood fibrin, will, with an equal dosage, produce a similar and equivalent physiological change. However the blood concentration returns to normal more quickly with our proteose than when Witte's peptone is used. This may be accounted for by the fact that Witte's peptone contains a large proportion of primary proteoses and peptones, as well as secondary proteoses, and primary proteoses are generally more toxic than deuteroproteoses.

The hemoglobin percentage is taken as the measure of the relative blood concentration of the dog. The values given in the charts are relative ones, the hemoglobin being expressed in percentage of the initial readings rather than the absolute value. The dosage employed in both series was 0.2 gm. of the protein per kilogram of body weight. The proteins were rapidly injected intravenously. The intravenous method is the route par excellence for the therapeutic use of hemo-proteose, and has been the method of clincal choice in practice. This obviates the increasing

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[5] Underhill, F. P., and Ringer, M., The relation of blood concentration to peptone shock, *J. Pharm. and Exp. Ther.*, 1923, xix, 163.

TABLE I.

The Toxicity of Brooks' Hemo-proteose for Albino Rats. (Intraperitoneal Injections.) Date, February 19, 1924.

No. of rat.	Wt. in gm.	Dosage in mg. per 100 gm. of rat.	Liquid dose per 100 gm. of rat.	Total no. cc. injected.	Total wt. of protein injected in gm.	Time of injection A. M.	Time interval until death in minutes.	Time of death A. M.	Symptoms.
1.	191	5	0.5 cc. of 1%	0.95 (1%)	0.0095	9:21			None
2.	177	10	1.0 cc. of 1%	1.77 (1%)	0.0177	9:24			Cyanosis
3.	234	20	2.0 cc. of 1%	4.68 (1%)	0.0468	9:26			Cyanosis
4.	250	40	0.8 cc. of 5%	2.00 (5%)	0.100	9:27			Cyanosis
5.	277	60	1.2 cc. of 5%	3.32 (5%)	0.1662	9:28			Cyanosis
6.	217	75	1.5 cc. of 5%	3.25 (5%)	0.1627	9:29			Cyanosis
7.	181	100	2.0 cc. of 5%	3.62 (5%)	0.181	9:31			Convulsions
8.	229	125	1.25 cc. of 10%	2.86 (10%)	0.2862	9:33	24	9:57	Convulsions
9.	190	150	1.50 cc. of 10%	2.85 (10%)	0.285	9:36	32	10:08	Convulsions and shock
10.	254	175	1.75 cc. of 10%	4.44 (10%)	0.4445	9:38	17	9:55	Convulsions and shock
11.	197	200	2.00 cc. of 10%	3.94 (10%)	0.394	9:39	11	9:50	Convulsions and shock
12.	203	225	2.25 cc. of 10%	4.97 (10%)	0.4567	9:41	13	9:54	Convulsions and shock
13.	231	250	2.50 cc. of 10%	5.77 (10%)	0.5775	9:45	8	9:53	Convulsions and shock
14.	233	275	2.75 cc. of 10%	6.40 (10%)	0.640	9:47	20	10:07	Convulsions and shock

non-absorption of the proteins when injected subcutaneously, which is caused by precipitation at the locus of injection.[6]

At present the quantity of proteose used for intravenous injections for the human varies from 15 to 40 mg. repeated every forty-eight hours. The administration of this small quantity is quite safe. The lethal dose for the albino rat is 125 mg. per 100 gm. body weight. Applying the lethal dose for the rat to man, 87.5 gm. would be the lethal dose for a 70 kilo man. This is more than two thousand times the largest therapeutic dose employed. (See Table I.)

CONCLUSIONS

1. The so-called "reaction" seen in protein therapy is not at all necessary in order to secure beneficial therapeutic action.
2. Shattered hemo-protein has qualities which make it useful in therapy: it is desensitizing; it acts as a non-specific immunizing antibody; and it is of low toxicity, producing almost no "reaction" in therapeutic doses.

166 (2398)

The differentiation of two distinct types of phagocytic cells in the spleen of the rabbit.

By R. S. CUNNINGHAM, F. R. SABIN and C. A. DOAN.

[*From the Department of Anatomy, the Johns Hopkins University, Baltimore, Md.*]

It has come to be the accepted opinion that the phagocytic cells of the tissues, which have been designated as *clasmatocytes, macrophages* or *histiocytes,* and the large mononuclear elements of the blood, the so-called *monocytes* or *transitionals,* are cells which are very closely related if not identical. The majority of observers consider them to be derivatives of the reticulo-endothelial apparatus or of endothelial cells in general; indeed one of us (Sabin[1]) has presented the view that they are identical cells derived from endothelium. A few observers have maintained that there are differences in the two types but even they accept a common ancestry from endothelium or from a hypothetical histiogenic stem-cell. Many observers have noted the great abundance of phagocytic cells in the spleen and have assumed that the spleen is especially favorable for their production.

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[6] Opie, E. L., The fate of injected protein in an animal immunized against it, PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 162.

[1] Sabin, F. R., *Contrib. to Embryol.*, 1920, ix, No. 36, Carnegie Institution of Washington, Publication No. 272.

With this concept in mind, we have studied the living cells which can be obtained from the spleen by puncture and have come to the conclusion, that, however difficult it may be to establish criteria for two distinct types of phagocytic cells in fixed tissues, in living material definite and satisfactory criteria can be established for separating these cells into two distinct types.

The spleen of an anesthetized rabbit was exposed, a capillary pipette plunged into the substance of the organ, and the blood and tissue cells thus obtained were studied in supra-vital films. The stain used for this study was a combination of neutral red and Janus green.[2] With this technique, it soon became apparent that in addition to the lymphocytic and leucocytic strains of white cells which were always present, the remaining cells could be sharply differentiated into two groups. One group consisted of cells to which the names *clasmatocyte, macrophage* and *histiocyte* have been applied. They were usually large cells, 15 to 30 micra in diameter. These cells always reacted intensely to neutral red; the aggregations of the stain varied greatly in size and color (light red to deep maroon) and were scattered irregularly, without any pattern whatever, throughout the cell. Many of these cells contained phagocytized erythrocytes and occasionally leucocytes; the red blood cells, which had been ingested, sometimes had the color of hemoglobin but in other cases were much darker in tint. The smaller cells always resembled the larger in regard to the character and distribution of the dye and phagocytized material; when only a single cell had been taken up it was always placed adjacent to the nucleus on the side where the greatest amount of cytoplasm was found. The nuclei were usually oval in shape, peripherally located, and relatively small in comparison to the size of the cell. These cells contained very few mitochondria; the larger cells had none at all or only a few which showed a faint reaction to Janus green; the smaller cells, however, quite frequently contained a moderate number of definite mitochondria scattered throughout the cytoplasm.

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[2] It is a pleasure to state that samples of both these dyes, which have been obtained from the National Aniline and Chemical Company, have been found wholly satisfactory. Their neutral red is a pure stain and must be used in a much more dilute solution than the Grübler dye.

The other group consisted of cells which could be identified with the typical monocytes of the peripheral blood. These cells were almost always uniform in size, about 15 micra in diameter, and their horseshoe or kidney-shaped nuclei were large in comparison to the amount of cytoplasm. These cells stained quite characteristically in neutral red. Opposite the hof of the nucleus there was a central clear spot surrounded by a rosette of small radiating aggregations of neutral red, the most peripheral of which were considerably larger than the others. The characteristic color of the neutral red enclosures of the monocyte approximated a soft salmon tint, and there was never the wide variation in color-shading which has been described for the clasmatocyte. The mitochondria, which were likewise always present, were brilliantly stained in Janus green and were located in the periphery of the cell. This appearance of a central, clear area with a rosette of small granules radiating from it, surrounded by the larger neutral red bodies, and by the mitochondria, seems to be quite specific for the monocyte and is usually retained up to the point of cell disintegration. The rosette is occasionally obscured temporarily when the cell is in active motion but it can be seen to reappear when the cell resumes a round or flattened-out shape; here the distinctive reaction to neutral red helps to identify it. This cell is also phagocytic and the reason that its pattern is so persistent is that the material which is phagocytized is placed in the zone of the larger neutral red bodies on the periphery of the rosette.

In spleens in which no myelocytes could be found and, where there were no evidences of a myeloblastic reaction, cells were found in large numbers showing every transition between the full-grown monocyte and a characteristic primitive cell containing only mitochondria. This transition between the primitive cell and the adult monocyte is marked by the appearance of a clump of a few, fine neutral red granules among the mitochondria. These granules never disperse throughout the cytoplasm as is the case in the development of the myelocyte, but remain close to the clear spot or the centrosphere and very early assume the characteristic radiating arrangement noted above. It is also important to note that these neutral red granules never become so numerous as in the developing myelocyte nor so varied as in the clasmatocyte. In the case of the monocytes of the spleen it has been comparatively rare to find one which has phagocytized whole cells, though occasionally we have seen a monocyte containing one or two red blood corpuscles; in these instances it has been striking that the phagocytized cell was peripheral to the rosette, while in the case of the other type of phagocytic cell (the clasmatocyte) the first red blood corpuscle to be taken up was placed in the area comparable with the center of the rosette of the monocyte.

Therefore these two types of cells present this contrast, that, while the one, the clasmatocyte, shows an absence of pattern both in the reaction to neutral red and in the position of the phagocytized cells; the other, the monocyte, has a striking and persistent pattern in its staining with the neutral red. Differential counts of many normal spleens indicate roughly the following average percentages: monocytes 17 per cent, clasmatocytes 1 per cent.

Our observations lead us to conclude that the monocyte arises from a stem-cell of mesenchymal origin which is resident in the pulp, where, according to the observations of one of us, (Cunningham[3]) there is no endothelium whatever; while on the other hand the larger, more phagocytic cell, the so-called clasmatocyte or histiocyte, is a derivative of the endothelium of the sinuses. The question of terminology is a difficult one since all of the terms used have been applied to both types of cells; we retain the term *monocyte* for the type of cell which occurs in the blood-stream and restrict the term *clasmatocyte, histiocyte* or *macrophage* to the endothelial derivative of high phagocytic power.

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[3] Cunningham, R. S., unpublished observations.

167 (2399)

The separation of the phagocytic cells of the peritoneal exudate into two distinct types.

By F. R. SABIN, C. A. DOAN and R. S. CUNNINGHAM.

[*From the Department of Anatomy, the Johns Hopkins University, Baltimore, Md.*]

The observations reported in the preceding communication[1] led us to consider the possibility that the phagocytic cells occurring in peritoneal exudates might be separated into the same two groups that were found in the spleen. It has been possible to demonstrate that this is true. A normal rabbit was given intraperitoneally 25 cc. of whole blood, which had been removed by a sterile puncture from the heart of another rabbit, and, after 24 hours, samples of the exudate were removed and studied. Another injection was then given and the examination repeated at the end of the second 24 hours. This procedure was repeated at 24 hour intervals over as long a time as was desired. Some of the experiments were carried out on rabbits whose blood-cells had been carefully matched, while in other instances animals were selected in which the serum of one agglutinated the red blood-cells of the other and *vice versa.* The exudate was studied with neutral red and Janus green in vital-dye films. It has been shown[2] that the first reaction that takes place after the introduction of any substance into the peritoneal cavity is the emigration of polymorphonuclear leucocytes, which appear in the exudate at the end of four to six hours. In the material obtained from our animals at the end of 24 hours there were always large numbers of leucocytes, the majority of which were actively motile, though some were rounded up and a few were in the "non-motile phase." In addition to these, the predominant cell was the typical clasmatocyte which had phagocytized erythrocytes and whose general appearance corresponded very closely with those previously described.[1] They were usually large cells, varying in size from 20 to 30 micra in diameter. At this stage only part of them contained phagocytized red cells, the remainder being filled with large and small neutral red vacuoles of various sizes and shades of color. These clasmatocytes were entirely characteristic, with oval nuclei, extensively vacuolated cytoplasm, few or no demonstrable mitochondria, and no radial arrangement of the neutral red around a centrosphere. At this stage there was always great variation in the amount and character of the exudate. In some experiments with matched blood, only clear fluid with few or no cells was obtained after 24 hours and at times even after 48 hours (*i. e.,* after two injections); while with unmatched blood there was generally a considerable amount of sero-sanguinous exudate at the end of 24 hours. With unmatched blood we found a few monocytes at 24 hours but they were not very numerous until 48 or 72 hours, when they had largely replaced the clasmatocytes and made up about 60 per cent of all the cells and about 90 per cent of the phagocytic mononuclears. These monocytes were entirely comparable to those seen in the spleen, except that they had been stimulated to much greater activity. They varied from cells the size of leucocytes to the medium sized clasmatocytes. These monocytes invariably contained a group of neutral red granules which radiated about a central clear area and this arrangement persisted even with the most active phagocytosis, cells having been found with from four to six red blood-cells and occasionally a leucocyte in the periphery of the cytoplasm, but never in the area of the rosette. The monocytes were in every stage of stimulation from the small cells similar to those of the peripheral blood to larger cells with heavy granules of red about the centrosphere and several phagocytized cells stored in the periphery. The mitochondria were characteristically distributed about the nucleus and the rosette; they were more numerous in the less stimulated cells, but were always more numerous in the monocytes than in the clasmatocytes. The clasmatocytes have not been seen to divide; but the monocytes have been observed to divide actively. The latter were seen in all stages of division and we have watched many of them actually divide on the slide. With a more chronic irritation, six to eight days, the monocytes were still predominant but there were more clasmatocytes than at the 48 to 72 hour intervals.

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[1] Cunningham, R. S., Sabin, F. R., and Doan, C. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 326.

[2] Cunningham, R. S., *Am. J. Physiol.*, 1922, lix, 1.

It seems therefore assured that the phagocytic cells of peritoneal exudates, which are induced by the exhibition of whole blood, can be separated into two distinct groups of cells; one corresponding to the clasmatocytes or histiocytes of the spleen and connective tissues and the other to the monocytes of spleen and circulating blood. In all of these experiments we have found no evidence of any transformation of serosal lining cells into other types of cells; whenever seen, the serosal cell is entirely characteristic whether in a normal, stimulated, or degenerating condition. We have also made a few observations on the subcutaneous tissue after the introduction of blood, on the omentum and on the phagocytic cells obtained from the lung. In general here also we have been able to differentiate the same two types of cells.

168 (2400)

Clinical experience with the active principle of cod liver oil.

By HUGH CHAPLIN.*

[*From the Department of Pediatrics, College of Physicians and Surgeons, Columbia University, New York City.*]

Between the end of December, 1922, and the end of May, 1923, an attempt was made to determine the anti-rachitic activity in infancy of the active principle of cod liver oil[1] prepared in the Department of Pathology at the College of Physicians and Surgeons.

For this purpose two similar groups of infants were observed in the Out Patient Department of Bellevue Hospital. All the infants in one group received the active principle, those of the other serving as controls. In general each child had a monthly physical examination and an x-ray of the wrists. Their homes were visited regularly and classified *one, two, three* or *four* according to the number of rooms, how many were light or dark, and how much the infants were out of doors.

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* Introduced by T. F. Zucker.

[1] Zucker, T. F., Pappenheimer, A. M., and Barnett, M., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 167; Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 136; Zucker, T. F., and Matzner, M. J., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 186.

The active principle was administered in tablet form and, later on, dissolved in olive oil, the latter being the method of choice because the tablets, though active when fresh, sometimes lost some of their power, possibly because of drying.

All judgment as to the presence of rickets or healing is based on x-ray findings.

The following results were obtained: The homes of the control group were equally divided between classes *two* and *three* whereas those of the treated group were one and one-half times as many in classes *three* and *four* as in class *two*. In both the treated and control groups there were from two to three times as much rickets in classes *three* and *four* as in class *two*.

Sixty per cent of the treated group had rickets and only 35 per cent of the control group. Ninety-four per cent of all the rickets cases in the treated group showed healing and showed it so early as to be directly contrary to the season's expected influence. Sixty-six per cent of the rickets cases in the control group showed healing but so late as to be entirely accounted for by the season's influence.

Thirty-seven per cent of the non-rachitic cases in the treated group showed increased calcification and but 9 per cent of the controls.

It is believed that these facts prove beyond a doubt that the active principle of cod liver oil, already shown to be active in rat rickets, is also active in infantile rickets.

169 (2401)

Observations on the mechanism of complete heart block in man.

By ARTHUR C. DE GRAFF and SOMA WEISS.

[*From the Department of Electrocardiography of New York University Medical College and from the Second (Cornell) and the Third (New York University) Divisions of Medicine of Bellevue Hospital, New York City.*]

Observations were made in eight cases of complete heart block; and the findings in five cases, admitted previously to Bellevue Hospital, were used as material. Over 300 electrocardiographic tracings of one minute duration were analyzed.

Epinephrine was selected as a stimulant for the sympathetic end apparatus in the heart. Subcutaneous doses of 1 to 2 mg. increased the ventricular rate considerably. This increase persisted from 15 minutes to several hours. The auricular rate and the blood pressure showed no, or moderate, increase. Change in the auricular rate did not go parallel, as a rule, with the change in the ventricular rate. The experimental findings suggest that the ventricles have direct and rich sympathetic nerve supply.

Atropine sulfate was administered in doses of 5.0 mg. and 3.5 mg. respectively. The results of these observations indicate that the influence of the vagus on the sinus node is the same in complete heart block as in normal individuals. The effect of atropine on the auricular rate lasted over three hours. The maximal increase was reached as early as 10 minutes and as late as 50 minutes after the injection. The ventricular rate was slightly but distinctly increased, indicating that the vagus exerts a slight inhibitory influence on the ventricles in complete block.

If a certain amount of epinephrine, which increased the ventricular but not the auricular rate, was given after the vagus had been paralyzed; there was an increase in the rates of both the auricules and the ventricles.

Following and probably during exercise there was a marked increase in the auricular rate. The ventricles showed but slight increase as late as 15 to 25 minutes after exercise.

The normal ventricular rate in the cases studied varied from 9 to 84 per minute. The average auricular rate was 78, the average ventricular rate was 31 and the average blood pressure 162/67. Except at the beginning of the establishment of block, the latter could not be changed to normal mechanism either by exercise or by chemical stimulation.

Tracings in a case of right-sided preponderance showed repeatedly that following the injection of epinephrine the right-sided preponderance changed temporarily to left and then again to right. The time relations of premature ventricular contractions were studied.

170 (2402)

Binaural minimum audition in a subject with ranges of deficient acuity.

By A. G. POHLMAN and F. W. KRANZ.

[*From the Department of Anatomy, St. Louis University, St. Louis, Mo., and the Wallace Clement Sabin, Laboratory of Acoustics, Riverbank, Geneva, Ill.*]

While working on the problem of the bone transmission of sound, the writers found several peculiar effects. At certain pitches, pulsating ringing tones were heard synchronous with the heart beat. Other frequency ranges showed evidences of diplacousis. In determining in which ear a sound was apparently being heard, when the sound was being applied to both ears, "cross-over" effects from one ear to the other occurred as the pitch was changed without referable cause in so far as the acuity curves for the two ears was concerned. Inasmuch as bone transmission is necessarily conveyed to both ears, it was thought essential that binaural acuity for air transmitted sounds be tested as well as monaural. It was also considered important to find out whether or not any reinforcing effect due to the use of the two ears which might be shown in the ranges of normal hearing, would similarly be found in abnormal ranges.

The type of apparatus used has been described elsewhere.[1] Alternating electrical currents were generated by means of a vacuum-tube oscillator and sounds were produced from these currents by the use of the thermophone. The frequencies and intensities were regulated by electrical means. For binaural tests, two exactly similar thermophones were used in series, so that the measured current ran through both of them and the same intensity of sound was applied to the two ears. The absolute intensities were calculated from the currents and from the characteristics of the thermophones.

The results obtained are shown graphically in the accompanying sketches where the sensitivities are given for each ear and also both together. The difference between any two adjacent horizontal lines, as 8 and 9, represents a factor of 10 in the intensity of the applied sounds. The matter of attention introduces a certain factor of variability in the results, perhaps amounting

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[1] Kranz, F. W., Minimum intensity for audition, *Phys. Rev.*, 1923, xxi, 573.

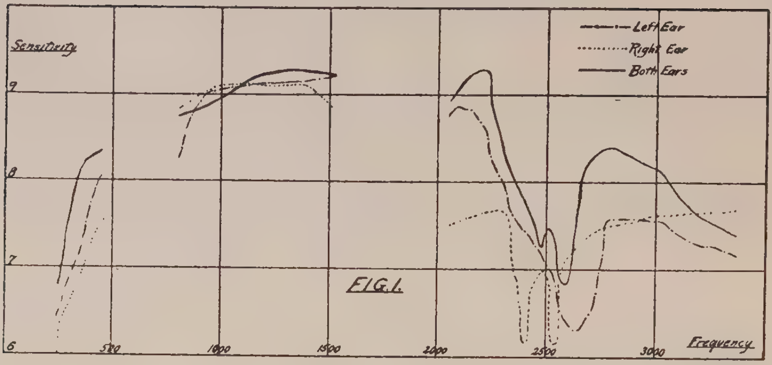

FIG. 1.

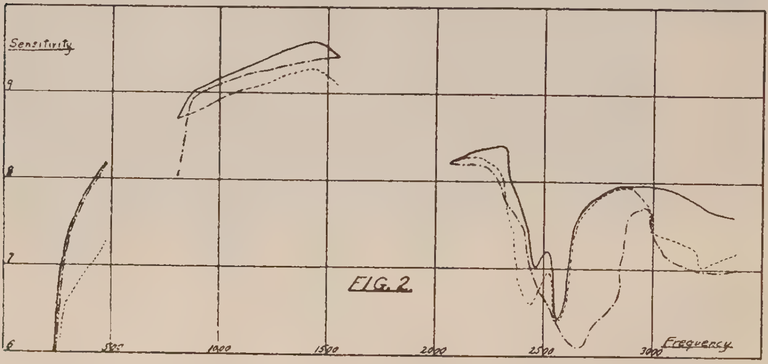

FIG. 2.

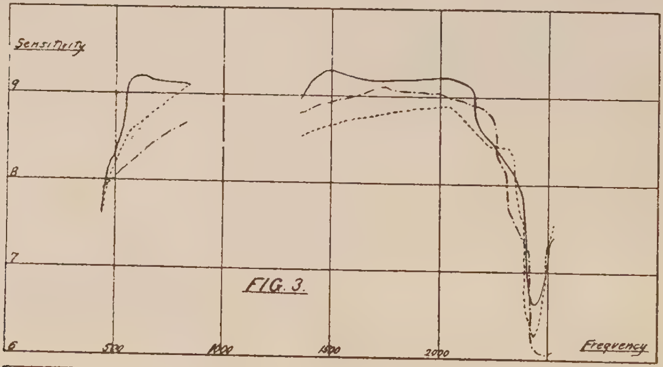

FIG. 3.

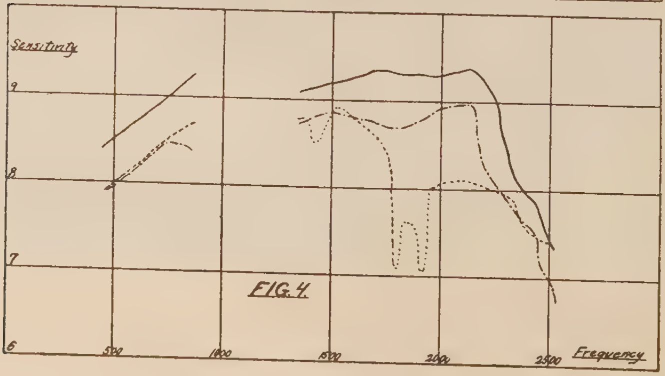

FIG. 4.

to 0.2 or 0.3 on the scale of sensitivity shown. The frequency is given in double vibrations per second. It is seen that binaural sensitivity is in general not much different from the sensitivity of the better ear but it is also, with few exceptions, slightly higher. It may be concluded that the binaural sensitivity at minimum audibility is but slightly better than the sensitivity of either ear alone, and this is also true in the ranges of depressed sensitivity as well as in the ranges of normal hearing.

It was found that while the region of decreased acuity was very noticeable for air transmitted sound near minimum audibility, the depression was not nearly so evident in the case of bone transmitted sound. The question came to mind—in passing over a frequency range, will a portion of this range which shows a low value of acuity when at or very close to the low limit of audibility, continue to be noticeably low in comparison with the remainder of the range as the intensity of stimulus for the whole range is increased? It was found by trial that when the intensity of the stimulus was about 10 times that required to hear the weakest part of the range, this low sensitivity portion was no longer noticeable as the frequency was varied to pass through it.

The conclusions in this paper may be summed up in two statements:

1. The sensitivity for binaural minimum audition is consistently slightly higher than for either ear alone. The amount, however, is not large, and in fact, does not lie far beyond the limits of a reasonable error in attention.

2. Decreased acuity of hearing for certain limited portions of the tone scale as determined by minimum audible sounds, is not a criterion of deafness with respect to ordinary intensities. Once the threshold of such a range of decreased acuity is attained, it takes comparatively little additional energy to cause the deficiency to seemingly disappear quite completely.

171 (2403)

Some favorable effects from the alimentary administration of insulin.*

By JOHN R. MURLIN, C. CLYDE SUTTER, R. S. ALLEN and H. A. PIPER.

[*From the Physiological Laboratory, University of Rochester and the Park Avenue Clinical and Highland Hospitals, Rochester, N. Y.*]

Since July, 1922, when absorption of insulin from the alimentary tract was first observed by us [1, 2] various attempts have been made to devise a practicable method for oral administration. No difficulty was experienced in demonstrating absorption of simple extracts by depancreatized dogs proving that insulin can survive the destructive action of the stomach.[3] With normal animals, normal human subjects and diabetic patients having an efficient supply of trypsin the insulin was almost always destroyed.[4, 5] Nevertheless a few instances of obvious absorption were encountered in human diabetics. These were reported in brief at the 11th International Congress[6] of Physiologists at Edinburgh, July, 1923. In this same month attempts were made to delay the destructive action of trypsin by combining with insulin certain weak organic acids and enclosing the combination in enteric coated capsules. Later enteric coated tablets were prepared for us by reputable pharmaceutical houses. Several cases have now been treated for different lengths of time with these tablets. Not all reacted favorably.

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* Presented at the meeting of the Western New York Branch of the Society for Experimental Biology and Medicine, Rochester, N. Y., February 16, 1924.

[1] Sutter, C. C., and Murlin, J. R., Proc. Soc. Exp. Biol. and Med., 1922, xx, 68.

[2] Murlin, J. R., *Endocrinology*, 1923, vii, 519.

[3] Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stokes, A. M., *J. Biol. Chem.*, 1923, lvi, 253.

[4] Dudley, H. W., *Biochem. J.*, 1923, xvii, 376.

[5] Witzemann, E. J., and Livshis, L., *J. Biol. Chem.*, 1923, lvii, 425.

[6] No abstract of this report was published because it was received too late.

TABLE I. Tests on Human Cases.

Patient	Date	Amt. and No. of Extract.	Drop in blood sugar mg.	Hrs.	Drop in urine sugar in 24 hrs. gm.	Remarks
		Duodenal Administration. 1st blood before breakfast.				
	1922					
Bu[1]	July 6-7	360 cc. No. 25 (2) + 0.1 per cent HCl	61	11	22	
	8-9	400 cc. No. 33 + 0.2 per cent HCl	40	2⅓	20	
	15-16	250 cc. No. 39 (1) + 0.1 per cent HCl	40	2	34	Following 250 cc. 0.3 per cent Na_2CO_3
	18-19	500 cc. No. 41 (2) with 6 per cent alc.	60	4	8	
	20-21	1000 cc. No. 49 (1) with 6 per cent alc.	60	4¾	34	2 doses 3½ hrs. apart
Bl	13-19	500 cc No. 45 (2) with 8 per cent alc.	101	6	16	Patient intoxicated
	22-23	500 cc. 5 per cent alc. alone	36	3	7	A. M.
		500 cc. No. 48 (2) with 5 per cent alc.	42	5½	13	P. M.
	25-26	700 cc. No. 46 (1) with 5 per cent alc.	53	8½	6	
	27-28	1000 cc. 5 per cent alc. alone	34	8½	1.1	
	29-30	800 cc. No. 46 (1) no alc.	42	9¼	5.0	
		Stomach Administration of Enteric Coated Tablets—constant diet.				
	1923		Blood sugar level mg.			
E. V.	Nov. 8	30 units insulin subcut. and 6 tablets daily	50		sugar free	
	9	30 units insulin alone	142		sugar free	
	1924				sugar exc. 24 hrs. gm.	
E. M.	Jan. 7-12	6 to 12 tablets daily Jan. 7	500		61.6	Both blood and urine had been on increase previous to use of tablets.
		steady drop to Jan. 12	265		13.7	
	15-19	16 tablets daily Jan. 15	344		42.7	
		steady drop to Jan. 19	303		19.8	

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[1] This case is reported from Highland Hospital by the courtesy of Dr. John R. Williams.

Two of those giving the best reactions are shown in the table together with several additional tests by duodenal administration not previously reported.

In all of the four cases reported the patients were on constant diets. The results show: (1) that insulin contained in 0.1 or 0.2 per cent HCl is absorbed from the human intestine; (2) that addition of alcohol to 5 to 8 per cent increases the absorption somewhat; (3) that alcohol alone, however, produces a drop in blood sugar but only a slight effect on the 24 hour elimination of sugar by the kidney; (4) that insulin combined with a weak organic acid or acid salt, the whole contained in an enteric coating which survives the stomach for at least three hours, can be absorbed in sufficient amount to lower blood sugar and urine sugar very materially. Failure to produce such effects in some cases seems to be due to retention of the tablets in the stomach until they are finally destroyed.

Other means of delaying the action of trypsin temporarily are now being tried.

ABSTRACTS OF COMMUNICATIONS.

Minnesota Branch.

Sixteenth meeting.

Minneapolis, Minnesota, February 16, 1924.

172 (2404)

The antiscorbutic value of canned vegetables and its preservation by exhausting the air before processing.

E. L. SCHIELD (by invitation).

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*]

Commercial canned corn, beans or peas contain considerable antiscorbutic vitamin. By removing the air before processing, the antiscorbutic vitamin is doubled or quadrupled. This work was completed a year ago.

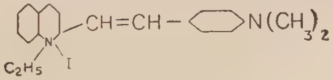
173 (2405)

Determination of gastric acidity and pH.

By J. F. McCLENDON.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

The free HCl of the gastric contents may be easily titrated using brom phenol blue as an indicator. The "total acidity" has no chemical meaning. The pH may be determined by means of the Duboscq colorimeter, using quinaldine red



as an indicator. The centrifuged gastric contents are measured and an equal measure of distilled water taken as a standard.

The same amount of indicator is added to each. The percentage dissociation is determined by the percentage color. The pH is read from the following table:

Percentage dissociation	2	3	4	5	6	7	8	9	10
pH	1	1.2	1.3	1.4	1.5	1.6	1.65	1.7	1.74
Percentage dissociation	14	20	24	29	34	39	44	50	
pH	1.9	2.1	2.2	2.3	2.4	2.5	2.6	2.7	
Percentage dissociation	56	61	66	71	76	80	91	99	
pH	2.8	2.9	3.	3.1	3.2	3.3	3.7	4.7	
Percentage dissociation		56	61	66	71	76	80	91	99

(The conductance ratio of HCl and the activity of the hydrogen ions is not exactly the same.)

174 (2406)

The effect of secretin on blood forming tissue.

By JOSEPH T. KING (by invitation).

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

It has been claimed by some[1] that injections of secretin stimulated the blood forming tissue. After injections of an acid extract of intestinal mucous membrane (secretin) there is a sharp rise in the blood count. The time required for the rise and return to normal is about three hours.

Using stained smears and supravital staining (brilliant cresyl blue) as criteria of new cell formation, it is found that there is no increase in the percentage of reticulocytes and no evidence of unusual marrow activity in the stained smears. Therefore, the rise in count must be due to an escape of plasma and not to increased cell formation.

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[1] Downs and Eddy, *Am. J. Physiol.*, 1918, xlvi, 209.

175 (2407)

Influence of age of parent culture on size of cells of Bacillus megatherium.

By ARTHUR T. HENRICI.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

In a previous communication[1] I have pointed out that during the early hours of a culture the cells of *Bacillus megatherium* become greatly increased in size and then rapidly decrease; that the increase in size is accompanied by increased variability, the frequency curves showing skewness and a tendency to bimodality; and that the degree of duration of the increase in size is greater with smaller seedings. These observations were made on cultures inoculated from a culture 12 hours old, *i. e.,* one which had already reached the maximum of growth. In the present study I have observed the variations in size in cultures transplanted from a parent culture after 2, 4, 6 and 8 hours of growth, *i. e.,* during the period when the cells were increasing and decreasing in size.

In the accompanying graphs the solid lines indicate the average length of the cells, the broken lines the coefficient of variation. The abscissæ indicate the age of the cultures in hours, the ordinates to the left length of cells in microns, those to the right coefficients of variation. The curves for the parent culture are repeated in the graphs for the various subcultures up to the time of transplanting.

When transplanted during the period of increasing size, at 2 hours, or at the time of maximum size, at 4 hours, the organisms continued to increase in size. It is noteworthy that the greatest decrease in size in these subcultures occurred between the 5th and 6th hours, as was also the case with the parent culture. The entire curves for these two subcultures, including the time spent in the parent culture, bear the same relationship to the parent culture as do curves obtained from cultures with small inoculums

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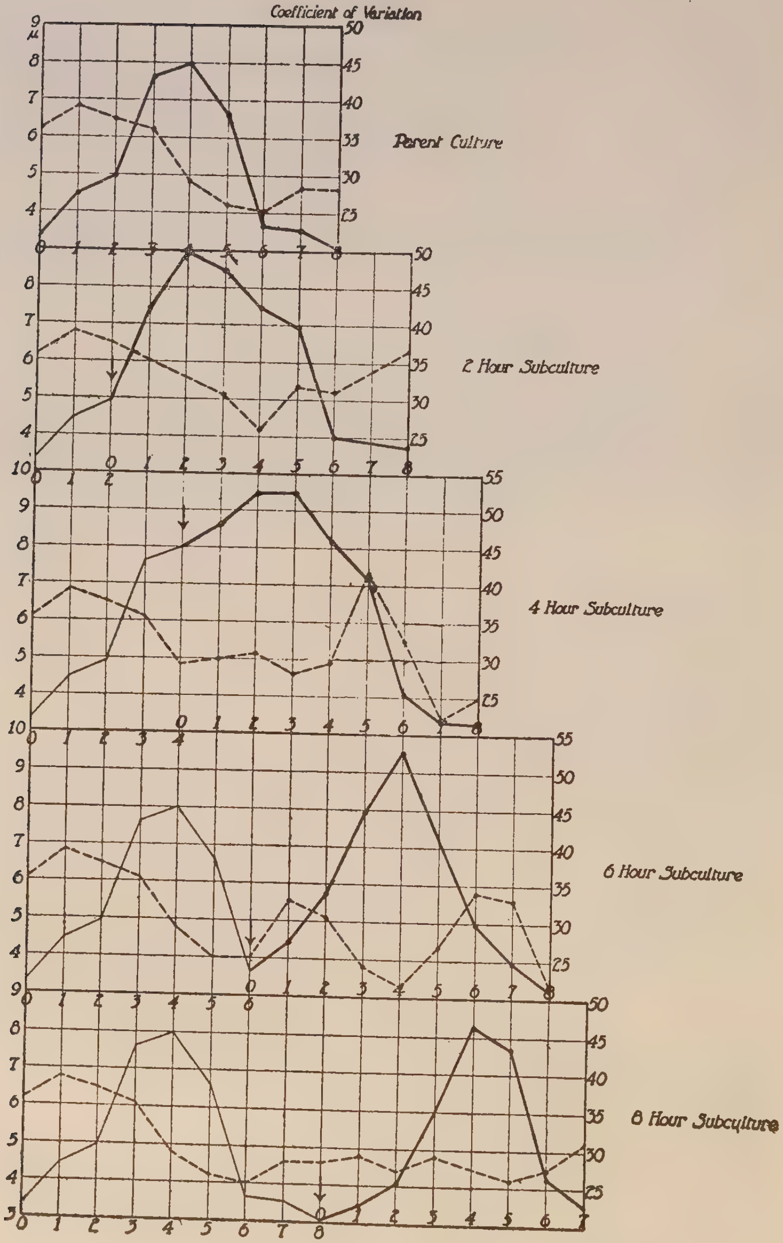
[1] Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 132.


Coefficient of Variation
9
μ
8
7
6
5
4
50
45
40
35
30
25
0 1 2 3 4 5 6 7 8
Parent Culture
2 Hour Subculture
4 Hour Subculture
6 Hour Subculture
8 Hour Subculture
10
55

to cultures more heavily seeded; there is a greater increase in size over a longer period of time.

The cells in the parent culture had practically decreased to the original size in 6 hours. Subcultured at this time they immediately increased rapidly again, reaching a higher maximum than did the parent culture. The eight hour subculture, however, showed an appreciable lag, and the curve is practically a repetition of that for the parent culture.

ABSTRACTS OF COMMUNICATIONS.

Seventeenth meeting.

Minnesota Branch.
Minneapolis, Minnesota, March 5, 1924.

176 (2408)

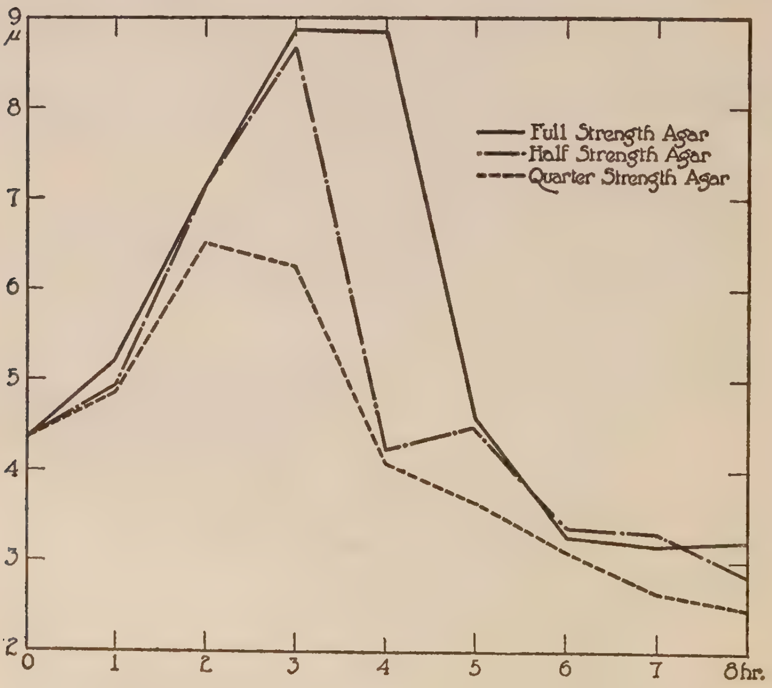
Influence of concentration of nutrients on size of cells of Bacillus megatherium.

By ARTHUR T. HENRICI.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

A young spore-free culture of *Bacillus megatherium* was inoculated on slants of standard beef extract agar, and on slants containing the same proportion of agar, but with one-half, one-fourth, one-eighth, and one-sixteenth as much of the nutrient ingredients, beef extract and peptone. The average length of the cells was determined at hourly intervals for eight hours. The results are shown in the accompanying graph. The curves for eighth-strength and sixteenth-strength agar were very similar to that for the quarter-strength, and have been omitted for the sake of clearness.

At the beginning of growth protoplasm is synthesized more rapidly than cell division occurs, and the cells become increasingly larger until a critical point is reached, when the reverse conditions obtain and the cells decrease to their original size. I have previously pointed out that this critical point is reached earlier, the maximum size attained being smaller, when the culture is heavily seeded than when small inoculums are used;[1] and that it may be postponed, the cells reaching a larger size, if a portion of the culture is transferred to a new medium before attaining their maximum length.[2] It would appear then that the size of the cells is determined, in part at least, by the density of the population, *i. e.,* the concentration of bacterial substance. But that this is not the sole factor is indicated by the experiment here reported; for there was much less growth in the mediums of lower concentration, yet the maximum length was smaller, and was reached earlier. Reducing the concentration of the nutrients has the same effect on the size-curve as increasing the concentration of bacterial substance, and the form of the curve is therefore determined by the proportion between these two factors.



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[1] Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 132.

[2] Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 343.

177 (2409)

Iodine metabolism: III.

By J. F. McCLENDON, J. C. HATHAWAY and LESTER NETZ.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

Statistically, both simple and exophthalmic goiter are related to iodine starvation. (Draft Board in U. S., Eggenberger in Europe.)

In iodine metabolism it is always necessary to analyze the food, but in making up diets it is convenient to know the approximate content beforehand. The following table gives food-stuffs prepared as for the table except that oats and barley contained the husk, which has the greatest amount of the iodine, but this may be eaten by some experimental animals. The prunes contained the pits which are not eaten. The loganberries contained the seeds which are not digested, therefore the iodine may not all be absorbed.

Milligrams of Iodine per Metric Ton of Dried Food (0—23 per cent H_2O).

	From Goiterous Region	Non-goiterous Region
Cereals	mg.	mg.
Wheat	1	3.5—8
Oats	10	20. —150
Corn		45. —220
Barley		63
Rye		3
Pot Herbs		
Spinach	18	
String beans	29	
Carrots	2	170
Soup vegetables	12.5	
Fruits		
Apples (peeled & cored)	2.5	
Pears (partly cored)	13	
Prunes	4.3	
Peaches (pits out)	10	
Apricots (pits out)	21	
Loganberries	139	
Salt (crude Great Salt L.)	1.2	
Animal Foods		
Skim milk	12	
Butter	140	
Goat milk		400
Salmon		45. —324

178 (2410)

The determination of the pH of the urine with 4 nitro 6 aminoguaiacol.

By J. F. McCLENDON.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

Since the "total acidity" of the urine has no chemical meaning it is often desirable to know the hydrogen ion concentration. At the suggeestion of Dr. J. B. Sumner, a sample of 4 nitro 6 aminoguaiacol which he kindly furnished, was used for this purpose. This appears to be a polybasic acid, which from pH 0 to 4 gives an increasing (yet always pale) yellow color, and from pH 4.5 to 8.5 gives an increasing henna color. The method is as follows:

In the left-hand cup of a Duboscq colorimeter place 0.5 cc. of an 0.1 per cent indicator, and 10 cc. of urine. In the right-hand cup place 0.5 cc. indicator, and 10 cc. 0.1 N NaOH solution. Adjust the left-hand cup to 20 mm., and hold under the right-hand cup a glass dish containing urine 20 mm. deep. Determine the percentage dissociation (percentage color) in the left-hand cup. In highly acid urines a color match cannot be obtained in this way. It is then necessary to place over the eye piece a glass dish with some of the indicator in 0.01 N HCl. The following table gives the approximate pH at different percentage dissociation:

pH	4.6	4.7	4.8	4.9	5	5.1	5.2	5.3	5.4	5.5
Per cent dissociation	3.0	3.5	4.0	4.5	5.5	6.5	8.0	9.0	11.0	13.0
pH			5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3
Per cent dissociation			15.0	17.0	19.0	22.0	25.0	29.0	33.0	37.0
pH			6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1
Per cent dissociation			42.0	46	50	54	58	62	66	70
pH		7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0
Per cent dissociation		74	77	80	83	85	87	89	91	92

ABSTRACTS OF COMMUNICATIONS.

Peking Branch.

Sixth meeting.

Peking, China, February 11, 1924.

179 (2411)

An experimental study of the local tissue reactions in the guinea pig to the Bacillus proteus x 19 as affected by the simultaneous injection of carbon.*

By RALPH G. MILLS, LI TING-AN and CHEN HUNG-TA (by invitation).

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

The relation of foreign bodies to the persistence of bacteria in tissues has been repeatedly observed from the clinical standpoint, but few studies of this relationship have been made. Organisms may remain active or latent in an area about a foreign body, or those which have invaded the circulation may localize there, initiating or reviving a suppurative process. The presence of such substances appears to protect organisms in their vicinity from the normal phagocytic activities of the body. The mechanism by which protection is afforded and the response of the organism to this protection, is the purpose of this study. This, in turn, offers a means by which to develop the "hypothesis of Welch", which emphasizes the importance of the changes undergone by bacteria in their conflict with the defensive powers of the host.

This investigation has no immediate relation to the question of the possible etiological importance of *Bacillus proteus* x 19 in typhus fever, but the organism was chosen because of the irregularity of its pathogenicity for guinea pigs, in the hope that conditions might be produced that would control this vagary and enable one to predict with certainty the outcome of a given experiment. The use of carbon was determined upon because of its recognized inert character and ready identification in the tissues. 1 cc. injection units of 10 per cent carbon suspension, bacteria from one-fourth of a 24 hour agar slant suspended in distilled water, or a mixture of the two, each of double strength, were introduced into the loose fascia of the back. The interaction of these two kinds of foreign bodies in the subcutaneous tissues of the guinea pig is outlined in the following summary.

1. Guinea pigs injected subcutaneously with a suspension of carbon show very slight general response and an extremely chronic local tissue reaction, with slow fibrosis of the injection site. Blockade of the lymphatics occurred early; and phagocytosis was moderate in amount, and associated with very little tissue destruction.

2. The injection of suspensions of *B. proteus* x 19 produced little or no reaction, with rapid widespread diffusion of the organisms, invasion of the blood stream and death from septicemia in about 35 per cent of the animals. If the infection were fatal, death took place within 24 hours; those animals surviving that length of time invariably recovered. Organisms were cultivated from the blood stream for two days and from the tissues for four days after injection, the blood thus becoming free from bacteria in a much shorter time. Histologically the bacilli were found in the tissues for at least 10 days, or 6 days after they could be recovered by cultural methods. Some of these organisms were free in the tissue spaces while others were intracellular. The majority of the bacteria were well preserved and displayed no evidence of disintegration. Their presence did not determine a progressively increasing leucocytosis, either locally or generally, during the period of their persistence.

3. Animals injected with carbon and proteus simultaneously, exhibited a local reaction similar to that observed in the carbon-injected animals, and a general reaction more like those receiving the proteus alone. The febrile reaction was a little less marked but persisted longer, and the loss in weight was more slowly made up. The initial death rate was 27.4 per cent, somewhat less than in animals receiving proteus alone, but the cause of death was the same in every case. The presence of the carbon seemed to protect a certain per cent of the animals from an otherwise fatal infection. The local reaction was in some instances more or less definitely inflammatory in character, and the size of the indurated area increased rather than decreased, as it had done in the carbon controls. In some cases the lesion softened and discharged its contents, in the pus from which a pure culture of *B. proteus* was found. Living organisms were thus recovered as long as 40 days after injection.

4. Carbon appears to exert a mild aggressin-like action upon the subcutaneous tissues, making it possible for these bacteria to maintain their existence and multiply locally in spite of the defensive mechanism of the animal body.

5. The serum of the carbon-plus-proteus animals agglutinated the organisms up to a dilution of 1-100, the same as did that from animals receiving the proteus alone, indicating that the presence of the carbon did not modify the agglutinative power of the serum.

6. The method developed has reduced the initial mortality of animals, after injection of *B. proteus* x 19, and has enabled the organisms to resist the bactericidal powers of the body for relatively long periods of time.

180 (**2412**)

The action of ephedrine, an alkaloid from Ma Huang.

By K. K. CHEN and CARL F. SCHMIDT.

[*From the Laboratory of Pharmacology, Peking Union Medical College, Peking, China.*]

Ma Huang, identified as *Ephedra vulgaris* var. *helvetica*, has been known to Chinese medicine since about 3100 B. C., when it was one of the herbs tested and approved by Emperor Shen Nung. According to the Pen Tsao Kang Mu (the Chinese Dispensatory, 1596) it improves circulation, causes sweating, stops cough, reduces fever, etc.

Nagai[1] obtained from the plant an alkaloid which he named ephedrine, and which he[2] found to possess a mydriatic effect. Amatsu and Kubota[3] described a rise in blood pressure and relaxation of the intestines after ephedrine. Merck[4] isolated from the European variety of *E. vulgaris* an alkaloid named pseudoephedrine isomeric with ephedrine.

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[1] Nagai, *Pharm. Z.*, 1887, xxxii, 700.

[2] Nagai, *Chem. Z.*, 1888, 755.

[3] Amatsu, H., and Kubota, S., *Kyoto, Igaku, Zosshi*, 1917, xiv, 77, through *Chem. Abs.*, 1918, xii, 2019.

[4] *Merck's Ber.*, 1893, 13.

In our preliminary investigation an intravenous injection of a simple extract of the crude drug to an anesthetized dog caused a sustained rise in blood pressure, accompanied by prolonged acceleration of heart and constriction of kidney vessels. An alkaloid was soon isolated from the drug and found to have the same effects. This alkaloid has been identified as ephedrine.

The injection of 0.25 to 10 mg. of the alkaloid into a vein of a dog or cat always causes a prolonged rise in blood pressure, cardiac acceleration, and constriction of peripheral vessels. In addition, intestines are relaxed and peristalsis inhibited, uterine tone is increased—these effects are the same in surviving tissues as in those in situ—pupils are widely dilated, and bronchial muscle is relaxed.

After the destruction of the brain and cord the drug acts as before, so that its effects are peripheral. A direct muscle action is eliminated by the tonic action on uterus and blood vessels and inhibitory action on gut and bronchi—effects that point to parasympathetic depression or sympathetic stimulation. That the parasympathetic is not paralyzed is shown by electrical stimulation of the vagus, for the weakest current that slowed the heart continues to be effective during the acceleration produced by ephedrine. Also, the pupil dilated by ephedrine, or the gut inhibited by it, is contracted by pilocarpine.

The most striking effects are those on the circulation. The myocardiograph shows greatly increased strength of contractions when acceleration occurs following the drug, but the isolated mammalian heart is only moderately stimulated by very dilute solutions and stronger concentrations cause pure depression. Application of the drug directly to the stellate ganglion causes a marked increase in rate and strength of contractions comparable to the first effect of local application of nicotine, but ephedrine does not paralyze after stimulating; after local application of nicotine to the ganglion, ephedrine applied locally has no effect, so that its action is not due to absorption from the pleura. The cardiac and blood pressure effects of application of ephedrine to the stellate ganglion are less marked than those of intravenous injection. Intravenous injection of toxic doses of nicotine does not prevent the typical ephedrine effects on heart and blood pressure in dogs.

The action of ephedrine on the heart appears to be (1) stimulation of accelerator ganglia; (2) stimulation of accelerator endings; (3) direct depression of heart muscle.

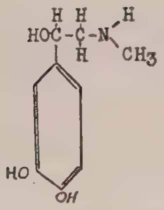
Peripheral vessels are constricted by ephedrine, but this effect is relatively weak and is often overcome by cardiac stimulation. The vessels of the perfused kidney show definite and prolonged constriction, while those of the perfused lung are affected only slightly. These effects are apparently not concerned with ganglia, for the volume of the kidney in situ is not affected by application of the drug to the thoracic ganglia, and after intravenous injection of nicotine ephedrine causes marked vasoconstriction.

The circulatory effects of one injection of ephedrine persist for 10 to 15 minutes. Repeated injections soon fail to cause further acceleration or augmentation of the heart, but a progressive rise of pressure occurs as a result of progressive vasoconstriction until a maximum is reached, often at a sustained pressure level of over 200 mm. Hg, even in a spinal animal. Beyond this, further injections cause pure cardiac depression.

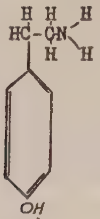
The other effects of the drug—on gut, uterus, pupil, bronchial muscle—are apparently due entirely to the stimulation of sympathetic endings. After nicotine the isolated gut is inhibited and relaxed by ephedrine, while pilocarpine stimulates after nicotine and ephedrine. The pupil is widely dilated when ephedrine is applied to the conjunctiva, while application to the superior cervical ganglion has no effect. The light reflex is not abolished by ephedrine, and pilocarpine action is not prevented. Bronchial spasm due to physostigmine is relaxed by ephedrine.

The action of ephedrine is therefore similar to that of adrenaline. Compared to adrenaline, ephedrine action is much more persistent; its cardiac effects are relatively stronger than its vascular action, which may be due to simultaneous stimulation of accelerator ganglia and endings; its action on the circulation soon reaches a maximum beyond which repeated doses fail to stimulate, and may cause depression.

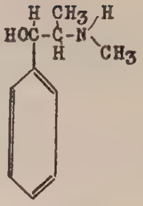
The similarity of the effects of ephedrine to those of tyramine and adrenaline is especially interesting in view of the similarity in chemical structure.



Adrenaline
Jowett[6]



Tyramine
Barger-Dale[6]



Ephedrine
Ladenburg[5]

It properly belongs to the group of "sympathomimetic amines" of Dale.[6]

The acute toxicity of ephedrine is low, the m.l.d. lying between 100 and 145 mg. per kilo in rats. These doses cause cocaine-like convulsions, which are probably the cause of death.

Further chemical and histological investigations of the plant are being made and a method of assay has been devised.

181 (2413)

The experimental transmission of leishmaniasis to animals.

By H. JOCELYN SMYLY and CHARLES W. YOUNG.

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

On December 18th, 1922, an autopsy (No. 3853) was performed on a patient who had died of kala-azar that day in the Peking Union Medical College Hospital. The spleen was found to be unusually heavily infected with *Leishmania donovani,* spleen smears showing hundreds of organisms in every field under the 1.9 mm. oil immersion objective. A portion of the spleen was taken, using sterile precautions, ground in Rosenow's tissue crusher, and mixed with 0.85 per cent salt solution to make an emulsion which easily passed through a fine hypodermic needle. The same suspension was used to inject the following series of animals, all of which were injected intraperitoneally except the dogs which were injected intravenously:

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[5] Ladenburg, A., and Oelschaegel, C., *Ber.*, 1889, xxii, 1823.

[6] Barger, G., *The Simple Natural Bases,* 1914.

2 dogs received 5 cc. and 4.5 cc. respectively; 2 guinea pigs, 0.5 cc.; 2 white rats, 0.5 cc.; 2 black mice, 0.3 cc.; 5 hamsters, 0.3 cc.

The hamster (*Cricetulus griseus* M-Edw.) is a species of field mouse which we describe more fully in another paper read at this meeting.

The results of these inoculations were as follows:

Dog 1—Blood culture positive on 38th day. Died after 5 months. No autopsy.

Dog 2—Blood culture positive on 42nd day. Autopsy after 4½ months. Spleen sections showed intracellular *L. donovani.*

Guinea pigs—Spleen smears negative at autopsy on 95th and 131st days.

White rats—Smears of spleen, liver, and marrow negative at autopsy on 29th and 90th days.

Black mouse 1—Autopsy on 111th day. Smears of spleen and liver positive. Sections of spleen, liver, and marrow show intracellular *L. donovani.*

Black mouse 2—Autopsy 121st day. Smears of liver and spleen positive.

Hamster 1—Autopsy 45th day. Liver and spleen smears positive.

Hamsters 2, 3, 4, 5—Autopsies on 8th, 116th, and 351st days. Smears of liver and spleen all positive. Sections of liver, spleen and marrow positive for intracellular *L. donovani.*

On August 25th, 1923, material from another autopsy (No. 4358) was used for the injection of another series of animals. This case was a very light infection, smears and cultures taken at autopsy from spleen and liver being negative for *Leishmania,* but smears from the bone marrow (rib and femur) being positive. Similar technique was used in the injection. The animals were all injected intraperitonially, their respective doses being as follows:

2 dogs with 10 cc. and 5 cc. of spleen emulsion; 2 dogs with 10 and 5 cc. of liver emulsion; 5 hamsters, 1 with 1 cc. spleen emulsion, 2 with 1 cc. liver emulsion; 2 with 1 cc. marrow emulsion respectively.

The resulting infections were as follows:

Dogs—All negative for *L. donovani* by liver puncture and sternum puncture on 102nd to 107th day. Still living.

Hamster 8—Autopsy 105th day. Spleen smear positive for *L. donovani.*

Hamsters 9, 11, 12—Liver punctures positive for *L. donovani.* Autopsy smears of spleen and liver positive for *L. donovani.* on the 116th to 144th days.

Hamster 10—Liver puncture on 131st day positive for *L. donovani.* Still living.

Inoculations into white mice were made from both autopsies, 13 mice being used; all died of injury, the Chinese white mouse being extremely small; no results were obtained for comparison.

Considerable attention has been given by other workers to experimental *Leishmania* infections in dogs, mice, and other animals. The point of chief interest in these studies is in the observation for the first time that the hamster *(C. griseus)* is easily infected with *L. donovani.* The minimum infective dose has not been determined, but the fact that animals were successfully infected by liver and spleen emulsion which was negative on microscopic examination of smears and on culture indicates a high susceptibility. The infection in these hamsters has run a chronic course without any apparent tendency to spontaneous recovery. One infected hamster lived almost a year, and was then killed as it looked sick and had an abscess in the cheek. As a rule they showed no apparent ill effect. At autopsy the spleen was always greatly enlarged, in one case as much as fourteen times the normal weight in proportion to body weight. Smears of spleen and liver showed *L. donovani* in enormous numbers, while sections of the organs showed the organisms in innumerable swarms within large cells probably endothelial. Passage of the virus was easily effected to other hamsters.

182 (2414)

Experimental kala-azar in a hamster.

(Cricetulus griseus, M.—Edw).

By CHARLES W. YOUNG, H. JOCELYN SMYLY and CABOT BROWN.

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

This paper is not intended to be an account of a completed problem. It is rather a progress report. On account, however, of the almost uniform success in infecting this hamster with kala-azar, it seems advisable to publish the data thus far obtained. Further search among related rodents may reveal a still more suitable animal.

The experimental study of kala-azar has been hampered by lack of susceptible laboratory animals. Those commonly used, such as the monkey, dog, white mouse and white rat are not uniformly infected even when the number of organisms injected is large. In 1919, Hsieh[1] introduced into use for pneumococcus grouping, a Chinese hamster *Cricetulus griseus,* M.-Edw. This rodent is found wild locally, and is easily and cheaply obtained. It thrives in captivity and is said to breed if earth is provided for burrowing. In another paper read at this meeting, we have described the experimental transmission of the North China kala-azar to animals. The first case (Hospital No. 3852) was a very heavily infected one. Among the animals inoculated were five hamsters, all of which became infected. The second (Hospital No. 4358) was a very lightly infected child. At autopsy the spleen and liver smears and cultures were negative. The marrow smears were positive. Of the hamsters inoculated and surviving the bacteria present in the material, all become infected with kala-azar, namely, one injected with spleen, two with liver and two with marrow. In the studies here reported the aflagellate form of *Leishmania donovani* or Leishman-Donovan body has been used. This was obtained, except the original inoculations, from mice or hamsters, usually the latter.

Experimental Work. Preparation of Material. The human material, spleen or liver was ground in a Rosenow tissue crusher, and suspended in 0.85 per cent saline or Locke's solution. In the case of that obtained from rodents, one liver and one spleen were ground as before and suspended, usually in 10 cc. of saline or Locke's solution and injected into 20 hamsters.

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[1] Hsieh, E. T., A New Laboratory Animal *(Cricetulus griseus). National Med. J. of China,* 1919, v, 20.

Intraperitoneal Inoculations. These were made with a sterile syringe carrying a No. 20 or 22 needle, both boiled in 0.85 per cent saline. The belly of the hamster was first sponged with alcohol. Examination as to whether the animal was infected, was made by liver puncture by a similar technique. In the latter case, gentle negative pressure was exerted by pulling on the piston of the syringe while withdrawing the needle. Intracellular organisms were found at varying intervals from 1 to 332 days after inoculation. The question arose in the earlier cases, whether the parasites found came from the liver indicating infection, or were from the unabsorbed inoculum. Twenty hamsters were injected each with 0.5 cc. of liver and spleen suspension from two other infected animals, and sacrificed four daily. In each animal, a study was made of smears from (1) centrifugalized peritoneal washings, (2) liver rinsed twice with saline to free it from the inoculum, (3) similarly washed spleen, and (4) bone marrow (femur). The intracellular forms persisted in the peritoneal cavity in large numbers throughout. Organisms appeared in the spleen (smears) first after 3 days, and in the liver and marrow after 4 days. The organisms in the liver were more numerous than in the marrow but the material used was much more suitable. In two other hamsters, 9 days after inoculation, intracellular parasites were found in the washings from the peritoneal cavity as well as in the washed liver and spleen smears. Peritoneal punctures were made on animals inoculated at varying periods from 28 to 334 days. The report of these will be given later. To summarize: 136 intraperitoneal inoculations have been made. One hundreds of these have been studied thus far. Omitting eleven negative cases of one, two and three day hamsters among the 20 noted above, the remaining 89 show the following results:

	Positive	Negative
Smears from organs	28	3
Liver punctures	51	5
Tissue sections	2	0
	81	8

This gives 91 per cent positive results from intraperitoneal inoculations.

Intrapleural Inoculations. Of two hamsters inoculated intrapleurally, one showed positive spleen smears fifteen days after inoculation. The second gave a positive liver puncture 53 days after injection.

Subcutaneous Inoculations. Eleven hamsters were inoculated subcutaneously. Of these four have been examined. One showed negative liver punctures after 22 and 28 days, but became positive after 53 days. The three others were autopsied 51 days after injection. One showed positive liver, spleen and marrow smears. The remaining two were negative.

Feeding Experiments. Five hamsters were starved 36 hours and then fed on the liver and spleen of an infected animal divided approximately equally among the five. Two died within eight hours and one other after seven days. No cause of death was found at autopsy. The two remaining animals were sacrificed 18 days after feeding. Liver and spleen smears were negative.

DISCUSSION.

The question of the earliest interval after intraperitoneal inoculation, at which liver puncture may be taken as a true criterion of systematic infection is still unanswered. While this is true it may be said that in no case in which early liver punctures were positive has the animal failed to show systemic infections at autopsy later. Our hamsters showed no tendency toward spontaneous recovery. The longer they lived the heavier the infection became. In one animal dying 325 days after infection (No. 7), the spleen weighed 1.52 grams, which is about 14 times the normal.

SUMMARY AND CONCLUSIONS.

A Chinese hamster (*Cricetulus griseus,* M.-Edw.) is very susceptible to inoculation with the aflagellate form of *Leishmania-donovani* when the organisms are injected intraperitoneally.

It is also susceptible to intrapleural inoculation.

It is sometimes but not uniformly infected by subcutaneous inoculation.

The two feeding experiments were negative.

183 (2415)

A comparative method for testing the enzymes of living hemolytic streptococci. I. Lipase.

By WILLIAM P. THOMPSON and FRANK L. MELENY (by invitation).

[*From the Department of Surgery, Peking Union Medical College, Peking, China.*]

Enzymes capable of digesting proteins, carbohydrates and fats have been demonstrated for the hemolytic streptococcus by Stevens and West who extracted these ferments from dead organisms by a process of grinding. This method is not available for quantitative determinations because of the difficulty of complete extraction by the grinding process. Nor can comparative determinations be made of different strains of the streptococci in culture media because of the variability of different strains in their rapidity of growth and because of the modifying factor of the media itself. It is possibible, however, by the use of "indifferent" suspending fluids such as Locke's solution with 0.1 per cent gelatin or a solution containing 1.0 per cent sodium chloride and 0.05 per cent calcium chloride with 0.1 per cent gelatin and by quantitative determinations of bacterial concentration by means of the Gates turbidimeter, to compare the ferment action of a single organism under varying conditions or of different organisms under identical conditions.

The usual method of determining lipolytic activity by other workers has been to titrate with sodium hydroxide the acid produced in the enzyme substrate mixtures. Dietz has shown, however, that enzyme preparations of different activity, as measured by their velocity constants, reach the same equilibrium point if allowed to continue to completion. Within certain limits, when there is an excess of substrate present, the velocity of the reaction is in direct linear proportion to the quantity of active enzyme present. These facts together with the difficulty of titrating minute quantities of acid produced by small quantities of bacteria suggested the possibility of measuring the velocity of the reaction between certain definite hydrogen ion concentrations.

Standard color tubes were prepared by taking up heat killed organisms, in the same concentration as in the test suspensions, with Clark's buffer solutions at pH levels of 8.0, 7.6 and 7.2 with phenol red as indicator. Then, under certain varying conditions, suspensions of living streptococci in the indifferent suspending fluids were allowed to act upon ethyl butyrate. In the active tubes the formation of butyric acid caused a more or less rapid change of color from pH 8.0 to 7.2. The rapidity of the change of color was taken as an indication of the activity of the lipolytic ferment under the given conditions.

With this method it was found that: (1) the acid production was more active in young cultures than in old; (2) there is an almost linear curve relationship between the speed of reaction and the concentration of the organisms; (3) the optimum temperature for the reaction is around 37.5°; (4) there is no action at 62° C. or above; (5) the property is destroyed by heating the bacteria to 60° C. or above for 10 minutes; (6) the optimum hydrogen ion concentration is around pH 7.8; (7) the activity is not increased by increasing the virulence of the organism by repeated rabbit passages; (8) the localization of the organism in the subcutaneous fat with a solution of this tissue in a certain clear cut clinical group of cases previously reported, is not due to a more active lipolytic ferment in the organisms recovered from these cases than is present in hemolytic streptococci from certain other clinically heterologous sources.

184 (2416)

The relation between the roots of plants and fungi.

By W. T. COUNCILMAN.

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

The relation of the roots of plants to the fungi in the soil forming a true symbiosis, is of importance not only in botany from the light it throws on some forms of plant nutrition, but in medicine also from the close relation between symbiosis and parasitism.

The roots of all annual plants and many others are closely related to the soil surfaces by fine hollow processes given off from the epidermic cells termed root hairs. While the leaves of the plant through the chlorophyl are the agents of production of the carbohydrates, the roots furnish the nitrogenous compounds and salts and the enormous amount of water which the plant requires. The roots of many of the shrubs and forest trees either have no root hairs or these are but slightly developed. This is especially the case in all of the ericaceæ and in most of the forest trees. The ericaceæ have very fine roots extending widely in the soil particularly in the surface humus.

The fine roots are accompanied by fungus hyphæ which extend upon the surface of the root, often forming a close surrounding mycelium, and certain of the hyphæ penetrate. The hyphæ on the surface are termed epitrophic and those penetrating endotrophic, but there is no sharp separation, both types being found in most plants. Some of these penetrating hyphæ extend along just beneath the epidermis, here and there sending short processes into the cells. The main cell invasion takes place from the hyphæ on the surface which sends fine haustoria into the epidermic cells, which by their extension and branching completely fill up the cells.

Much interest has been found in following the seasonal variation of the fungus relations. In the late summer and autumn in the *Epigaea repens,* the shrub most studied, there are great numbers of the invaded cells, the branching fungus within them giving a certain similarity to a kidney glomerulus. In the spring at the period of most active growth, the fungus filaments within the cells break up into a granular debris and disappear, the cell at the same time becoming more turgid. It seems probable that the fungus at this time contributes to the nutrition of the plant through its digestion by the intracellular enzymes, for the plant lays up no food supply. On the other hand the plant provides carbohydrates to the fungus during the active growth of the latter.

In the case of the forest trees such as the oaks, beeches, birches and all the coniferæ examined, the relation is different. All these trees have much coarser roots as compared with the ericaceæ, the latter having terminals often no more than 40 to 50 micra in diameter while the terminals of the forest trees are rarely less than twice this. They moreover terminate in a bulbous point which is to be regarded as a definite organ into whose structure both fungus and root enter. The fungal hyphæ extend along the root just as in the ericaceæ, but do not enter the cells though forming a close surrounding plexus. As the terminal is reached the fungal investment is closer, completely surrounding the root, and at the terminal bulb the outermost hyphæ form a dense, hard, black sclerotium with short branches having a more or less concentric arrangement. From this outstreaming short hyphæ all of about equal length, with no intercommunications and no septa, extend. These have no relation with the fungus in the surrounding humus. The fungus investment around the sclerotium extends between the epidermic cells which are literally buried in the fungus, but it does not extend into the parenchyma. Root hairs in these trees are entirely absent and the close relation to soil surfaces seems to be supplied by the hyphæ. In these trees also the relation of plant and fungus is one of mutual symbiosis, both being of equal dignity.

We are accustomed to think of the fungi as parasitic organisms but here there is no question of parasitism. Certain of these soil fungi are parasitic and pathogenic but most of them are not. There are certain conditions in which the plant becomes a parasite on the fungus as in the case of the *Monotropa uniflora* ordinarily known as the Indian pipe or corpse plant. This has no chlorophyl and is embedded in a fungus which forms a close investment over all underground parts and nourishes the plant during the brief period of growth, flowering, and fruiting.

Symbiosis has lately acquired great interest in medicine through the knowledge that certain organisms such as the Rickettsia may be symbionts in one organism such as the louse, and when transferred to another host may be pathogenic.

JACQUES LOEB

BORN APRIL 7, 1859—DIED FEBRUARY 11, 1924
President of the Society for Experimental Biology and Medicine 1916-1917

MEMORIAL TO JACQUES LOEB.

Jacques Loeb, the Man.

Jacques Loeb, savant, philosopher, mechanist, satirist, rebel, crusader, humanitarian—of whom shall I write these lines? The man is so big and still so near—too difficult to be embraced by a friend's eye, more so to be analyzed. I see his face, sunshine and shadows, smiles and frowns. How rapidly they interchanged, each capturing by its own force. Mankind and its weaknesses were his concern and the thoughts of these brought on the clouds, but to him, nothing was so sad as to be void of the humorous and thus the very grief begot amusement. And then there was Science, the guardian, the hope, the weapon!

I see Jacques Loeb at his desk in his modest home among his devoted family and surrounded by pictures of his spiritual friends, Voltaire, Diderot, D'Alambert. The men who dreamed of a realm where Reason was supreme authority, where all the crudities of superstition and outlived traditions were no longer permitted to crush human aspirations and human ambitions, where tolerance towered above all virtues. In them Jacques Loeb found his inspiration, their dreams became his dreams, their ideals he sponsored and to them he remained loyal and true through all his career and under all circumstances. It may not seem credible that a man who in his main pursuit was led by pure intellect should be moved by a motive emotional, should be inspired by the fire of a poet, by the zeal of a crusader. Nevertheless, such is the truth.

The efforts of Voltaire, Diderot and the entire group of encyclopedists were not in vain. The great French Revolution is the monument to their lives. But years passed and new tyrannies arose, new superstitions superceded the old and some of the old took a renewed hold. Times have changed and the methods of the old humanitarians no longer fitted the new conditions. Words, beautiful as they may be, eloquent as they may sound, were not the weapon Jacques Loeb chose to use in his struggle against superstition, in his crusade for intellectual freedom and for mutual tolerance. His ambition was to convince by realities hidden from the common eye. Thus, Jacques Loeb espoused Science, not merely as a career, nor as a medium to satisfy his personal curiosities. Science became his idol, his armor and his armament.

I see Jacques Loeb in his laboratory, again simple, surrounded by very few instruments and apparatus. From the walls are looking at him his old friends, Arrhenius, Van-t-Hoff, and Ostwald. Later the group was enlarged by Rutherford, Einstein and others. To them his eyes often turned for support and sympathy. It is here that the rebellious spirit inherited from Voltaire and fortified by revolutionaries in modern science found its full expression. Academic problems did not interest him; popular problems bored him. His weapon was always directed towards phenomena which had been surrounded by mystery and still more by superstition. In his quiet composed way, with tools as simple as toys, he gave battle after battle. As a Napoleon he tore to tatters the mantle of mystery from one phenomenon after another. His attacks were nearly always directed against phenomena which for generations had been considered supernatural—phenomena which Academies by tradition regarded as lying beyond the scope of human analysis. Thus, in one of his first endeavors, he threw his gauntlet to the old metaphysical philosophers and dared from the behavior of plants to explain the workings of the human will. Such was the origin of the work on tropisms and on the physiology of the brain. Later came other challenges. Parthenogenesis, regeneration, senescence; phenomena so much used in support of the ideas of vitalism were all divested of their mystery and presented in the simple light of rationalism.

Jacques Loeb realized the responsibility of his position. He anticipated criticism and antagonism. He realized that the more fantastic his ideas might seem, the saner must be the method which led to them. Thus, from the very beginning, his efforts were directed to support his doctrines by the methods of sciences which are the oldest and the most accurate; by mathematics, physics and chemistry. His true genius was in the discovery of simple systems in which life manifested itself in a simple way accessible to analysis by the methods of accurate sciences.

Jacques Loeb's energy was indomitable. He was always at work. His ideas were always ahead of his work. He was always planning. As years went on, his time was divided exclusively between his desk in the laboratory and his desk at home; books and experiments in the laboratory, books at home. It seemed as if he had no recreation. And yet that was not so. A genius in work, he was also a master in the matter of relaxation. If Jacques Loeb had not been a great scientist, he might have become a great humorist. His gift for the discovery of the comical was extraordinary and moments of greatest preoccupation were often interrupted by a smile or a hearty laugh. In such moments he was full of truly Voltairian jeu d'esprit, and fortunate was the one who met him in that mood.

Iconoclast, rebel, fighter, Jacques Loeb had a happy life. His love of man was so deep, his sympathies were so true and so frankly expressed, his friendships, once established, were so carefully guarded, that affection flowed towards him from every direction. His personal correspondence was voluminous; his personal contact was sought alike by great and small, by old and young. The influence of this contact is inestimable. Yet his greatest affection was for Science. It was equalled only by his affection for his wife and for his children. In return, in the dark and troubled days that followed the year 1914, it was Science and his family that saved Jacques Loeb from despair.

I see Jacques Loeb again, when his eyes were closed in eternal sleep. All lines on his face had disappeared, worry and care were gone. There was a smile on his lips, gentle, kind and friendly. This parting smile is his life's reward.

P. A. LEVENE.

Jacques Loeb, the Scientist.

The death of Jacques Loeb at the zenith of his career comes as a great shock to all who knew him. His loss will be keenly felt not only among biologists but among men of science throughout the world.

I shall not here attempt to disclose the qualities which quickly made him a recognized leader in biology or the personal traits which endeared him to an ever widening circle of devoted friends. It need only be said that his goodness is an open book to all who knew him and his greatness will loom even larger with the lapse of time.

Of his influence on biology it would be difficult to give an adequate account. All his fellow-workers felt its spell and his death will not impair it: many who never knew him will be unconsciously guided by it in ways little guessed.

His conceptions were often so bold and original as to startle conventional thinkers. Fearless in attacking difficult questions of fundamental importance, he showed almost uncanny insight into the most obscure and baffling matters. His discoveries often had a dramatic quality in their unexpected and beautiful solutions of perplexing problems. His results were reached by methods so simple as to compel admiration. His papers were always clear, cogent, and convincing. It is no wonder that he was a powerful stimulus to his fellow-workers.

His mechanistic view-point profoundly affected biology and medicine and had an important influence on psychology and philosophy.

It is possible only to recall some important facts in his career, which to so many has served as example and inspiration.

He was born in Mayen, Germany, April 7, 1859. He attended the Askanisches Gymnasium in Berlin, 1877-1880, the Universities of Berlin, 1880, Munich, 1880-81, Strassburg, 1881-1885 (M. D., 1884; Staatsexamen, 1885), and in 1885-1886 he was again at the University of Berlin.

He was Assistant in Physiology at the University of Würzburg, 1886-1888, and at the University of Strassburg, 1888-1890. From 1889 to 1891, at the Naples Zoölogical Station, he laid the foundation for his work on marine biology which was afterward continued at the Marine Biological Laboratory at Woods Hole, Massachusetts, and at Pacific Grove, California. In 1891 he came to America and became Associate in Biology at Bryn Mawr College. From there he went to the University of Chicago as Assistant Professor of Physiology and Experimental Biology in 1892; later he became Associate Professor, and then Professor of Physiology. In 1902 he accepted the professorship of Physiology at the University of California. In 1910 he became Member of The Rockefeller Institute for Medical Research and remained there until his death.

Attracted in his student days by certain aspects of metaphysics, he became especially interested in problems connected with the freedom of the will. The idea that certain brain functions are localized in definite centers and that human conduct may be profoundly affected by disturbances in these centers led him to study medicine to gain the technical knowledge needed for experiments in order to learn to what extent apparently volitional acts can be controlled by physical and chemical agencies. These studies led him, before the age of thirty, to the revolutionary conception that the actions of animals may be largely explained on a physicochemical basis. He took from the botanist, Julius Sachs, the idea of tropisms and applied it to animals. He sought a mechanistic explanation of animal conduct which should drop the question of purpose and reduce the reactions of animals to quantitative laws. His researches in this field are summarized in English in two volumes, "Comparative Physiology of the Brain and Comparative Psychology" (1900) and "Forced Movements, Tropisms, and Animal Conduct" (1918).

He next undertook to control the growth and form of animals by physical and chemical means. The studies in this field, which he named "Physiological Morphology," covered a very wide range and continued to receive his attention up to the time of his death. The goal at which he aimed was to secure the same degree of control over living matter that the chemist and physicist have over their material and he felt that the best prospect of success lay in applying their methods to biology.

It was characteristic that in the pursuit of these researches he was prompt to utilize recent discoveries in physical chemistry, particularly those connected with the ionic theory and the theory of osmotic pressure. From 1897 on he published papers applying these theories to biological phenomena and they may be said to form the leitmotif of his subsequent work. Out of them arose, almost at once, two important discoveries, antagonism and artificial parthenogenesis. Both resulted from experiments of the simplest kind, carried out on marine organisms.

It was found that the fish, *Fundulus,* can grow and develop in distilled water but soon dies if sodium chloride is added. The addition of other salts, particularly those of potassium and calcium, which are themselves toxic when they alone are present, produces a harmless solution. Loeb called this a balanced solution, that is, one in which the toxic effects of one substance are offset by the antagonistic action of other substances. This conception proved to be a fruitful one.

As the result of subsequent experiments he concluded that these facts may be accounted for by the effects of the antagonistic substances on the permeability of the protoplasm. It may be added that his experiments on permeability, which covered a wide range, led to the conclusion that Overton's hypothesis is untenable.

His experiments on artificial parthenogenesis may be truly called epoch-making. In 1913 he reviewed them in a volume entitled "Artificial Parthenogenesis and Fertilization" (following the publication of two volumes on this subject in German). He continued work in this field for some years after the publication of this volume. He aimed at a complete analysis of the mechanism of fertilization, development, and heredity. His work on fertilization indicated that the principal function of the sperm in stimulating development is to carry into the egg a substance which produces a surface change leading to the production of the fertilization membrane. This effect could be brought about, independently of sperm, by a variety of physical and chemical agencies.

At the same time he succeeded in finding means to bring about crosses which never occur in nature. By a slight change in the composition of the sea water eggs could be fertilized by sperm of other species which could not normally enter the egg.

In the course of these studies a great number of questions presented themselves which excited his keen interest. The variety and extent of these problems can only be realized by a careful consideration of the contents of the volumes entitled "Studies in General Physiology" (1905), "Dynamics of Living Matter" (1906), "The Mechanistic Conception of Life" (1912), and "The Organism as a Whole" (1916).

Among these subjects may be mentioned the rôle of oxygen in metabolism, toxicity, and development. Another in which he was deeply interested is the cause of natural death and the means of lengthening life. In the course of these studies he found that in certain cases low temperature prolongs life to a remarkable degree. In this connection it may be recalled that the first studies on the temperature coefficient of the heart beat and of the transmission of stimuli in nerves were made by his students.

Another investigation which he initiated, and which grew naturally out of these studies, showed that the electric potentials existing in the organism can, in many cases, be accounted for qualitatively and quantitatively by relatively simple means, and that they can be imitated to a considerable extent by artificial models.

As he truly said, many biologists accept a mechanistic explanation of various functions of the organism but fail to employ mechanistic conceptions in dealing with the larger problems of organization and adaptation. It was, however, precisely these problems which fascinated him and he did not hesitate to attack them from his point of view. He showed that many characteristics of the organisms which are regarded as adaptive may be explained on a mechanistic basis. He felt that here, as elsewhere in biology, progress requires quantitative investigation and with this in view he began the quantitative studies on regeneration upon which he continued to work until his death. His latest book, *"La nature physiochemique da la régénération"* (Paris, 1924) is devoted to this subject.

His work began, as was said, with questions concerning the freedom of the will. But he found that in order to study these he must attack the simpler problems involved in the behavior of lower organisms. These in turn required for their understanding a study of the physical and chemical reactions on which they are based. It is, therefore, not surprising that in the closing years of his life he came to devote his attention almost wholly to the properties of colloids, upon which life phenomena so largely depend. In a recent volume on "Proteins and the Theory of Colloidal Behavior" (1922) he contends that the behavior of colloids may be explained by the ordinary laws of chemistry without recourse to theories based on adsorption. As in earlier researches he had found a clue to the solution of many problems by applying the theories of electrolytic dissociation and osmosis, so in the work of his later years he discovered a guiding principle in the theory of the Donnan equilibrium. By applying this he was able to give quantitative explanations of some of the most important properties of proteins and to reduce them to simple mathematical laws. These studies, important for chemistry as well as for biology, form a fitting termination of his activity.

Thus closed a career rich in the joy of pioneer adventure in fresh fields of thought, abounding in brilliant discoveries, and splendidly stimulating far beyond the boundaries of biology. It will always stand out as a prominent feature of the progress of biology toward the status of an exact science. It is a career which reveals everywhere a creative imagination and capacity found only in minds of the highest order.

W. J. V. OSTERHOUT.

Jacques Loeb's Work in General Biology.

"If it be true that the fundamental problem of physics is the constitution of matter, it is equally true that the fundamental problem of physiology is the constitution of living matter. . . . Nothing has retarded the progress of physiology and pathology more than the neglect of comparative physiology." (*Physiological Archives,* II, 1898, University of Chicago Press.)

"General physiology is identical with an energetics of vital phenomena." (*Arch. ges. Physiol.,* 1897, lxix, 249.)

These words of Loeb define the standpoint that he maintained consistently during a period of intense scientific activity that never slackened from the date of his first publications about 1886 to the day of his death. The breadth and simplicity of his fundamental convictions prevented him from becoming enmeshed in narrow problems or enslaved by technique. The energetics of vital phenomena might be studied equally in the problems of heliotropism and animal behavior, in physiological morphology, in parthenogenesis and fertilization, in the rôle of salts in the physiological economy, or in problems of protein and colloid chemistry. Whatever problems he attacked he approached with simplicity and directness, avoiding adventitious and superficial phenomena; he created working hypotheses as tools for the problem in hand, to be abandoned when they had served their function as tools, and often long before heavier footed critics had ceased their attacks. He thus appeared in field after field of biological work for a longer or shorter time, and never thereafter was such a field cultivated in the same manner as before. He was a dynamic force in biology; and fortunate we are in America that that force was exercised in our midst.

His longest institutional connection was the semi-official one that he held at the Marine Biological Laboratory at Woods Hole, with which he was connected from 1892 to 1923 (save for his residence of eight years, 1902-1910, in California) as investigator, head of the Department of Physiology (1893-1902) and

Trustee (1897-1923). It was here that he exercised his greatest personal influence on the body of American biologists through his contacts, dear to him and to his associates; he was a man of many friends, and his memory will be cherished as one of the most brilliant and beloved of the Woods Hole biologists.

As one of his first pieces of work Loeb devised measurements of mental activity that were among the first in this field (*Arch. ges. Physiol.,* 1886, xxxix, 592). He then became interested in the heliotropism of animals and its agreement with the heliotropism of plants (1890), thus laying the foundation of his tropism theory of animal behavior, a theory illuminated by numerous studies by himself and pupils extending over many years and culminating in his book, "Forced Movements, Tropisms, and Animal Conduct" (1918, J. B. Lippincott Co.). Students of animal behavior all over the world became divided in two schools, for and against Loeb's theory. It is safe to say his influence in this field of work has never been exceeded.

Before coming to Woods Hole in 1892 Loeb had published two series of studies on the "physiological morphology" of animals. He continued these studies there, along with work on animal behavior, in the early nineties, and published papers on heteromorphosis, on polarization, on the mechanics of growth, on the artificial production of double and multiple monstrosities in sea-urchins, and on the physiology of color patterns. In these studies his aim was to attempt to show in every case the connecting link between the chemical processes and the formation of organs. He was much influenced by Sachs' theory of specific formative substances and did not hesitate to apply it, for example, in explaining the localization of stolons and hydranths in the growth of hydroids, and later in the regeneration of plants. However, he never attempted to apply this theory wholesale to the explanation of the development of all the organs and characters that belong to the species. In the case of the origin of the "tiger-like" markings on the yolk-sac of *Fundulus,* for instance, he attempted to show by experiments that migration of chromatophores to blood vessels filled with circulating blood caused the formation of the specific color pattern.

Loeb's numerous studies in physiological morphology never in fact led to any consistent theory of large aspects of developmental processes. But they uncovered an important series of new facts and helped to open up fields of work in which others reaped results. The really important theoretical contribution was negative—away from preformation and deterministic theories and towards a physiological conception of the organism in all stages as a moving physiological equilibrium. This was a lesson especially needed to be learned by morphologists in the years of Loeb's greatest period of activity in this field. Loeb's influence on the subject of the physiology of development was thus sound and stimulating.

The discovery of artificial parthenogenesis by Loeb in 1899 at Woods Hole deserves perhaps to rank as the most important of his single discoveries. Directed by his early studies on balance of salts, and guided also to a certain extent by previous studies of Morgan and Mead, Loeb was led to the idea that something in the constitution of the sea-water prevented the unfertilized eggs of marine animals from developing parthenogenically; specifically suggested by previous experiments on rhythmical contraction were changes in the composition of the sea-water by reduction of the Na- and Ca- ions, or increase of the Mg- or K-ions, or both. He found in fact that sea-water containing a certain added quantity of hypertonic magnesium chloride brought about complete parthenogenetic development of sea-urchin eggs exposed to its action for about two hours. This result and the theory were published in the *American Journal of Physiology*, October, 1899, and April, 1900.

Now a very striking thing is that the theory was entirely wrong, in spite of the fundamental discovery made by its application, as Loeb himself showed the same winter in California and the following summer in Woods Hole. He found that by simply increasing the osmotic pressure of the sea-water, even by non-electrolytes, essentially the same effects could be produced, and he therefore concluded that parthenogenetic development is caused by the egg losing a certain amount of water. Later experiments in which he produced a much more normal type of development, by the successive action of a cytolytic substance and a hypertonic solution on the egg, brought him to still a third theory to which he adhered firmly, and which he defended in a long series of publications, and in two books summarizing his work in this field. The theory was essentially that in the activation of the egg, whether by the spermatozoon or by parthenogenetic agents, at least two factors are involved, namely, one which brings about a change in the surface of the egg (cytolysis) and a second, corrective factor which remedies an injury caused by the first factor. Even if this theory also is incorrect, as many believe, it does not detract in the least from numerous important discoveries in the parthenogenesis of other forms, in hybrid fertilization, and in the rate of oxidation in the egg, which he put forward in a long series of papers through the next fifteen years.

The discovery of artificial parthenogenesis aroused an immense amount of interest, both in popular and scientific circles. Confirmation came rapidly from many sources; the principle was extended by other workers to numerous other animals. Other theories and a considerable polemical literature directed against Loeb's theories arose. It proved in fact one of the most fruitful and stimulating discoveries in general physiology in many years. The history of the problem of artificial parthenogenesis illustrates in fact better than any of Loeb's discoveries the extraordinary catalytic influence that Loeb exerted upon the biology of his time.

These necessarily fragmentary sketches of Loeb's attack on certain of his problems will be supplemented by others. They illustrate some of the characters that made Loeb a great scientific genius: his unwavering adherence to his great conception of the development of an energetics of vital phenomena, his insatiable scientific curiosity, his remarkable scientific imagination, his use of the working hypothesis in all specific problems merely as tool, the simplicity of his hypotheses and of his technique, the charm of his personality, and the indefatigable, unconquerable spirit of the man.

FRANK R. LILLIE.

Jacques Loeb and the Rockefeller Institute for Medical Research.

Jacques Loeb spent fifteen fruitful years at The Rockefeller Institute for Medical Research. He organized the Division of Experimental Biology, which later became the Division of General Physiology. During the entire period he was head of the division and the mainspring from which its many publications emanated.

Loeb's removal from the University of California, with its nearby marine laboratory, to New York, where his work was carried on in an inland city, necessitated considerable readjustment of his problems. This change was to a certain extent mitigated by providing him with a special laboratory at Woods Hole where he spent the spring and summer months. Indeed, the return to Woods Hole, and thus to the environment and to the friends of his early productive period in America, was a particularly happy circumstance of his settling in New York.

In the selection of the particular group of problems to meet conditions in New York, Loeb was very fortunate. That this was the case could hardly have surprised his colleagues who were of course acquainted with his exceptional resourcefulness. While all the major subjects which Loeb investigated successfully and with rare insight during the forty years of devoted and strenuous activity are strung, as it were, together on a single luminous thread, yet it is noteworthy that the problems he set himself to solve in the last years of his eventful life prove to have been of remarkable significance.

In no real sense is there a break in continuity in the entire series of Loeb's investigations. From the beginning he was absorbed in the elucidation of the fundamental properties of living matter, and at a very early period he became imbued with the belief that means and methods alone were wanting to account for many of the phenomena on a basis of physical and chemical actions.

Thus it came about that, starting his career as a physiologist, he soon broadened into an experimental biologist and then into a leader in physical chemistry. He enriched all these fields, discovered new facts, compelled new interpretations, and was followed by a devoted and ever enlarging number of students and disciples.

But Jacques Loeb meant far more to the Rockefeller Institute than brilliant investigator and a fertile discoverer. He was to all—old and young investigators alike—an inexhaustible source of stimulus and suggestion, the value of which cannot be estimated. In spite of his habitual deep absorption in his own problems, he was accessible to all comers in search of aid which perchance he might render. In the midst of an eager and ever shifting daily group in the lunch room, his mind burned with exceptional brightness, and whether in one of his more serious or more playful moods, the interest of his table talk was ever the same. His conversation was devoid of commonplaces and, whether directed to scientific or to more secular subjects, it was notable for its penetration and its liberalizing qualities.

Loeb's pregnant mind reached out into fields of science remote from the borders of physiology even in its wider sense. He was at home in many branches of physics and chemistry, and had mastered higher mathematics to serve the ends of his ever expanding field of experimental exploration. His capacity for concentration was phenomenal; his productivity at once the wonder and the despair of his associates; and his fertility and versatility showed no sign of flagging up to the very end of his rich and useful life. It may be of value to note a circumstance of Loeb's working habits. He was as little behind with the writing out of his results, as he was in the mental operations which carried him inevitably and without delay into the new problems which engaged his interest. And thus when the end of his vivid life came suddenly, it found his last experimental investigation complete and in final manuscript form.

It may be said of Jacques Loeb that he was as truly beloved for himself as admired for his extraordinary scientific attainments.

SIMON FLEXNER.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF COMMUNICATIONS.

One hundred and thirty-ninth meeting.

College of the City of New York, April 16, 1924.
President Jackson in the chair.

185 (2417)

The response of marine worms to unilateral tension of their musculature.

By A. R. MOORE.

[*From the Physiological Laboratory, Rutgers College, New Brunswick, N. J.*]

It has been shown that in *Lumbricus*[1] and *Nereis*[2] the anterior segments of the animal respond to passive bending of the posterior part, by active turning of the head in a direction parallel with the new orientation of the tail. This has been termed the homostrophic reflex. The receptors for the reflex are distributed throughout the animal but the efferent nerves are limited in their origin to the anterior 20 segments in *Lumbricus,* and to the first 2 or 3 segments in *Nereis.* The afferent impulses travel forward by way of the ventral nerve cord. Bending of the head end does not affect the direction of backward locomotion, hence the mechanism of the homostrophic reflex exhibits polarity. Similar observations[3] have been recorded for *Diplopods* and for the larvæ of *Tenebrio molitor.*

A systematic study has shown the homostrophic reflex to play a part in the behavior of a number of nereid worms of the Mediterranean, namely, *Eunice gigantea, Eunice siciliensis,*

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[1] Moore, A. R., *J. Gen. Physiol.,* 1922-23, v, 327.

[2] Moore, A. R., *J. Gen. Physiol.,* 1922-23, v, 451.

[3] Crozier, W. J., and Moore, A. R., *J. Gen. Physiol.,* 1922-23, v, 597.

Halla parthenospeia, Nephthis scolopendroides, Sthenelais dendrocepis, and *Psammolyce arenosa.* In these forms the homostrophic reflex disappears with removal of the head ganglia. In the case of very long forms, such as *Halla* (80 cm.), bending the body posterior to the middle is without effect, but if the bend is made 20 cm. back of the head, the latter promptly orients itself at right angles to the segments immediately behind it.

No evidence of homostrophy was found in the nemertine worm, *Cerebratulus marginatus,* nor in the flatworm *Yungia aurantiaca,* nor in the *Sipunculida.* It thus appears necessary to the occurrence of the homostrophic reflex that the ganglia of the central nervous system be numerous and extensively distributed as in the segmented animals.

186 (2418)

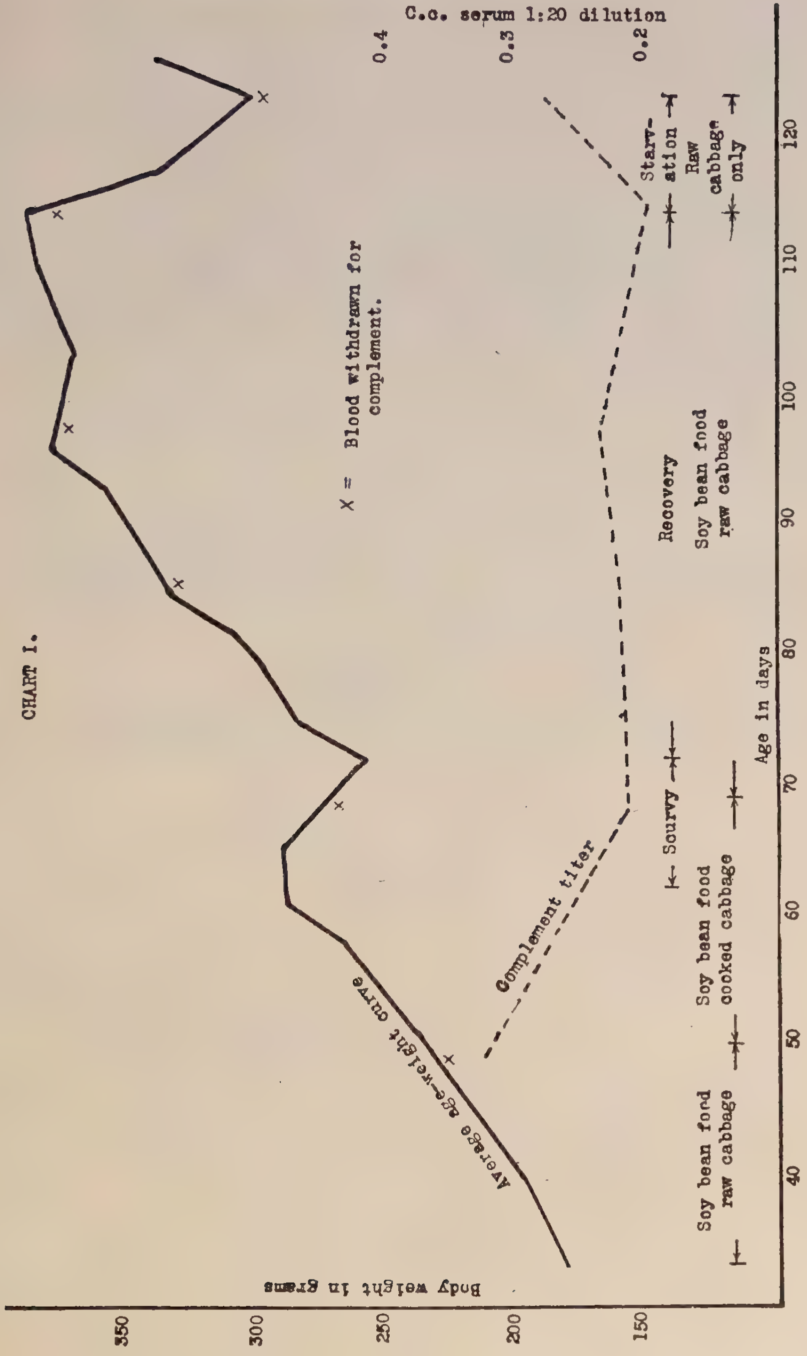
The variation of complement of guinea pigs during scurvy.

By MATHILDE L. KOCH and ARTHUR H. SMITH.

[*From the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

Since one of us (K) had observed that an intestinal infection markedly lowered the complement titer in guinea pig blood, it became of interest to follow the variation in titer throughout the development and cure of scurvy which, though not now classed as an infectious disease, is accompanied by a definite alteration in the blood vessel walls. The scurvy-producing diet consisted of soy bean meal (autoclaved) 86 per cent, dried yeast 3 per cent, sodium chloride 3 per cent, calcium lactate 3 per cent, and cod liver oil 5 per cent, to which was added 1.0 gm. per day dried boiled cabbage. Scurvy develops in 13 to 16 days in 200 gm. guinea pigs on this food, while merely changing from cooked dried cabbage to fresh cabbage causes this diet to suffice for normal growth in these animals.

The pigs were bled from the heart under light ether anesthesia, twice in the fore period when fresh cabbage was being fed, CHART I.


C.c. serum 1:20 dilution
0.4
0.3
0.2
X = Blood withdrawn for complement.
Average age-weight curve
Complement titer
Body weight in grams
350
300
250
200
150
Age in days
40
50
60
70
80
90
100
110
120
Soy bean food raw cabbage
Soy bean food cooked cabbage
Scurvy
Recovery
Soy bean food raw cabbage
Starv-ation
Raw cabbage only

once in the height of scurvy and again during recovery. One group of animals was then starved and bled after they had lost about 20 per cent of their weight. The effect of inanition during scurvy on complement was thus controlled. Clean, sterile apparatus and exceeding care in the volumetric measurements were emphasized.

From the chart it is seen that during scurvy a definite increase in the complement titer was observed over that in the fore period, while in the convalescent period the titer remained somewhat higher than in the fore period. Starvation resulted in a lowering of the titer. The effect of age, anesthesia and repeated bleedings are negligible according to Kolmer.[1]

We realize that the number of animals used (12) is small, but in view of the consistent and unexpected results we have offered them at this time. It appears from these experiments that there may be an obscure connection between the complement of the serum and the natural effort of the body to compensate for the tremendous physiological upset incident to scurvy.

187 (2419)

The oral administration of insulin to rabbits.

By HARVEY S. THATCHER.*

[*From the Department of Physiology, Columbia University, New York City.*]

Two papers have recently appeared on the absorption of insulin in alcoholic solution from the stomach. Winter[1] stated that insulin in weak alcoholic solution administered to rabbits *per os* caused the blood sugar to fall to a low level. Blatherwick[2] concluded that the oral administration of a weak solution of alcohol to rabbits caused marked decrease in the blood sugar. There

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[1] Kolmer, J. A., Matsunami, T., and Trist, M. E., *Am. J. Syphilis*, 1919, iii, 407.

* Introduced by H. B. Williams.

[1] Winter, L. B., *J. Physiol.*, 1923, lviii, 18.

[2] Blatherwick, N. R., Maxwell, L. C., and Long, M. L., *Am. J. Physiol.*, 1924, lxvii, 346.

TABLE I.

Date 1924	Rabbit No.	Wt.	Before injection	½ hr. after	¾ hr. after	1 hr. after	1¼ hr. after	1½ hr. after	1¾ hr. after	
			Glucose, mg. per 100 cc.							
Jan. 8	97	2400	130							1 cc. 95 per cent alcohol in 25 cc. H_2O. One additional cc. of 95 per cent alcohol was given each succeeding day in 20 cc. distilled H_2O, making 5 cc. on Jan. 12th.
Jan. 12	97	2400	158		154			111		5 cc. 95 per cent alcohol in 20 cc. H_2O.
15	97	2400	162	131						5 cc. 95 per cent alcohol in 20 cc. H_2O.
19*	97	2500	163	165			216	184		5 units of insulin in 21 cc. 20 per cent alcohol.
22	97	2500	160				136		135	100 units of insulin in 20 cc. 20 per cent alcohol (slight amount spilled).
7	56	2270	112							1 cc. 95 per cent alcohol in 25 cc. H_2O.
11	56	2300	122							One additional cc. of 95 per cent alcohol was given each succeeding day in 20 cc. distilled H_2O making 5 cc. on Jan. 14th.
14	56	2300	124	132		138		160		25 cc. of 20 per cent alcohol.
18	56	2515	116	148		160		170		2½ units of insulin in 20 cc. of 20 per cent alcohol.
20	56	2610	128	160		128				50 units of insulin in 20 cc. of 20 per cent alcohol.
Feb. 1	56	2600	154	138		108		98		0.89 units injected subcutaneously.
1	97	2500	166	77		82		84		0.875 units injected subcutaneously.

*Rabbit struggled.

was no absorption of insulin from the stomach even though large amounts were given in alcohol, whether it was dissolved in ice water or in 0.4 per cent sodium carbonate solution. There was a hypoglycemia with insulin in alcohol and with alcohol alone.

Recently, with the assistance of Levin, Allen, Malich, Nachamie, Knox and Hoff, members of the second-year class in physiology, we have carried out a series of experiments on administration of insulin in dilute alcohol. The Shafer-Hartman method for the determination of the blood sugar was used.

The rabbits were given the alcohol daily in increasing doses, thereby becoming accustomed to the stomach tube and to the narcotic effect of the alcohol. The animals were not fed in the morning before or during the experiments. The diet consisted of oats, hay and cabbage[3], a diet which should have rendered the rabbits sensitive to insulin[4].

The insulin used was the "Lilly U-20 units."

The results of these experiments are exhibited in the accompanying table.

CONCLUSION

While the results of our work are not conclusive, they make it seem improbable that insulin when administered in alcoholic solution is absorbed from the stomach in amounts that would give this method of administration any practical importance.

My thanks are due to Prof. E. L. Scott for suggesting this work.

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[3] Sherman, H. C., and Gettler, A. O., *J. Biol. Chem.*, 1912, xi, 323.

[4] Page, I. H., *Am. J. Physiol.*, 1923, lxvi, 1.

188 (2420)

Effects of sunlight on the malignancy of a transplanted tumor of the rabbit.

By WADE H. BROWN, LOUISE PEARCE, and C. M. VAN ALLEN.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

Since the tumor upon which this report is based was first transplanted in the spring of 1921,[1] we have been engaged in a study of the problem of animal resistance as related to the growth and malignancy of the tumor with especial reference to factors of constitutional resistance and conditions that might cause an increase or decrease in animal resistance.

Several reports have been published dealing with some of the results of this series of investigations.[2] Among other things, it has been noted that although a good primary growth can be obtained in practically all rabbits by intratesticular inoculation, the ultimate malignancy of the tumor is subject to wide variations and that, from the beginning, there has been a striking tendency to the occurrence of periods of excessive malignancy during the spring and fall of each year. This focused our attention on the possible influence of meteorological conditions and led to a study of phenomena of resistance and of tumor growth from the point of view of meteorological influences. The results of these investigations contain many points of interest, but the present report will be limited to a consideration of sunlight.

The method employed involved the inoculation of a group of 5 to 10 rabbits each month. Inoculations were made by injecting 0.2 to 0.3 cc. of a cell emulsion into one testicle. The animals were then kept under observation for a period of two months, at which time the surviving animals were killed. Records were kept covering the clinical course of the disease and all animals were subjected to a careful postmortem examination which included the weighing of organs, noting the number and distribution of metastases, and the extent and condition of the growth in all parts of the body. The data thus obtained were reduced to a form that would permit of a quantitative expression.

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[1] Brown, W. H., and Pearce, L., PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 201.

[2] Brown, W. H., and Pearce, L., *J. Exp, Med.*, 1923, xxxvii, 601, 631, 799, 811; *ibid.*, xxxviii, 347. PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 472. Pearce, L., and Van Allen, C. M., *Trans. Assoc. Am. Phys.*, 1923, xxxviii, 315.

When the results of these investigations were analyzed with reference to monthly and yearly records of meteorological conditions, as recorded at the Central Park Observatory, New York City, it was found that the malignancy displayed by the tumor at any time appeared to be closely related to the prevailing state of the weather and to sunlight in particular, as represented by the actual hours of sunshine. Thus, a comparison of the curve of sunshine with curves showing the incidence of metastases in different groups of animals and the relative distribution of the metastases brought out the fact that malignancy increased or diminished coincident with changes in the hours of sunshine. In general, it was found that the periods of maximum and minimum sunlight, corresponding with summer and winter, were periods of relatively low malignancy while the periods of greatest malignancy occurred at times of abrupt and rapid changes in the hours of sunshine, corresponding roughly with spring and fall. Still, the exact time of the occurrence of these periods of excessive malignancy was not the same in all cases. They occurred earlier or later, as the case might be, in conformity with the actual change in sunshine rather than with the regular progression of the seasons.

In like manner, minor variations in malignancy, occurring at other times, also coincided with changes in the curve of sunshine. In other words, almost every decided fluctuation in one set of curves was reflected in the other.

It appears to be certain, therefore, that even the diffuse sunlight of the laboratory is capable of exercising a profound influence on the malignancy of the tumor, or that some factor that is capable of influencing the curve of sunshine is equally effective in determining the course of this disease.

It also appears that while the prevailing level of sunshine is a factor of some importance in this connection, the factors of foremost importance are the rate, direction, and the extent of the change that occurs at any given time.

It is possible that the effects described may be due, in part, to an action on the tumor cells, but it is more likely that the malignancy of the tumor is influenced through an action on the animal organism. This conception is supported by the fact that, as reported elsewhere,[3] definite changes in the weight relationships of nearly all the organs of the body occurred in normal animals coincident with the changes in malignancy, and that the correlation was especially close in the case of organs that have been shown to be capable of exerting a profound influence on the malignancy of the tumor.

189 (2421)

Seasonal changes in organ weights and their relation to meteorological conditions.

By WADE H. BROWN, LOUISE PEARCE, and C. M. VAN ALLEN.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

It is well known that many of the endocrine glands of normal animals undergo rhythmic changes in weight per unit of body weight which conform, in general, with seasonal conditions. Still, the cause of these changes is not clearly understood nor is it generally known that other organs and tissues such as the heart, the liver, the kidneys, the spleen, the thymus, the lymphoid tissues, and even the brain, undergo changes of a similar character.

In November, 1921, we began a series of investigations which included among other things a study of the ratio of the weights of various organs and tissues to the body weight of normal rabbits and the limits of variation that might be encountered, with especial reference to the causes of such variations. The factors studied included age, breed, length of caging, time of killing (with reference to the regular system of feeding), and meteorological conditions. All of the animals used were males, and an effort was made to minimize the influence of dietary factors by maintaining a uniform system of feeding and a uniform diet consisting of hay, oats, and cabbage, which is the regular diet given all rabbits in these laboratories.

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[3] Brown, W. H., Pearce, L., and Van Allen, C. M., Seasonal changes in organ weights and their relation to meteorological conditions, PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 371.

The plan followed was to kill and examine two groups of rabbits each month selected from stocks that were being used for experimental work. All animals were well nourished and free from any external evidence of disease. During the first few months the groups were small and intentionally of a mixed character, but beginning with June, 1922, the monthly groups consisted of from ten to twenty rabbits.

The animals were killed prior to the daily feeding; they were anesthetized with ether and bled from the inferior vena cava while the heart was still beating. The organs were removed, freed of all adventitious material, and weighed on suitable balances. The weights were then reduced to terms per unit of net body weight which was determined by subtracting the weight of the gastro-intestinal mass from the live weight of the animal with an additional allowance made for excess urine in the bladder.

In this report we wish to refer briefly to one feature of the results obtained, namely, the seasonal occurrence of rhythmic changes in organ weights and the relation of the changes in certain organs to the monthly hours of sunshine as recorded at the Central Park Observatory in New York City. Other phases of the work will be reported later.

Without attempting to give a detailed statement of the results obtained, it may be said that all the organs examined were found to undergo rhythmic changes in weight per unit of net body weight which conformed, in general, to the progression of the seasons. In the case of organs such as the heart, the kidneys, and the liver, the transition from one condition to another occurred relatively slowly, and the maximum variation in any direction was distinctly less than that noted in the case of a number of the endocrine glands and the lymphoid tissues. The heart and the kidneys showed a variation in weight amounting to approximately 20 per cent while that of the liver reached upwards of 40 per cent.

The change in the weights of the endocrine glands, the thymus, the spleen, and the lymphnodes was much more pronounced and in some cases amounted to as much as 50 to 100 per cent.

On attempting to correlate this series of seasonal variations in organ weights with meteorological conditions, it was found that the majority of them corresponded in time and direction with prevailing conditions of sunlight. In general, the periods of maximum and minimum weights coincided with high or low levels of daily sunlight while the change in direction and the transition from one condition to the other corresponded with the change in sunlight from winter to summer or from summer to winter. What is more significant, however, is the fact that the actual time and progress of the change followed the curve of the actual hours of sunshine rather than the theoretical curve or the uniform progression of the seasons. This was more noticeable in the case of some organs than of others. In fact, it appeared that the weight curves of some organs conformed more closely to the curve of temperature or to humidity than to the curve of sunshine, and that the degree of correlation in any case was subject to the influence of other factors.

The most important deductions to be drawn from this series of observations are: First, that no fixed relationships exist between the various organs and tissues of the body—that mass relationships and probably functional activities are subject to continuous change in response to certain external influences such as are represented by climate or meteorological conditions; second, that these changes tend to pursue a rhythmic course in harmony with the progression of the seasons, but that they are retarded or accelerated or otherwise affected in accordance with prevailing meteorological conditions, and that there is a particularly close relationship between the state of the animal organism at any given time or the occurrence of a change in the relations that exist, on the one hand, and the prevailing condition of sunlight, on the other. Finally, in considering the influence of factors of this kind, there are two things to be taken into account, namely, the normal or prevailing level of activity and the factor of change.

190 (2422)

The use of calcium chloride in edema due to heart failure.

By HAROLD J. STEWART.*

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

This study concerns the reduction of edema in heart failure especially in those patients in whom the effort to increase the amount of diuresis by digitalis, theocin and diuretin fails. Blum[1] and his coworkers have made efforts in this direction, using calcium chloride as an agent and giving it by mouth. Singer,[2] basing his observations on Loewi's experiments, reported striking successes by giving calcium chloride and digitalis together intravenously. It may be recalled that Loewi[3] believes he has shown that calcium and digitalis have identical effects, the function of digitalis being merely to sensitize the heart muscle to the action of calcium. In one patient whom we have observed over a long period of time digitalis, theocin and diuretin produced a diuresis but not to the extent of freeing the patient of edema. She was, on the other hand, on repeated occasions made edema free by using calcium chloride. We did not make studies to determine the mechanism by which this diuresis was brought about. The results are summarized in Table I. Obviously calcium chloride produces diuresis both when given alone and when combined with digitalis. At times the diuresis continues from one to two days after calcium chloride has been discontinued. Of a total of 16 observations in which calcium chloride was given by mouth to five patients only once did it fail to produce some degree of diuresis. In two patients the diuresis resulting from the calcium chloride was effective in freeing them of edema,

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* Introduced by Alfred E. Cohn.

[1] Blum, L., and Schwab, H., L'action du chlorure de calcium, dans les hydropisies cardiaques. Les dangers de l'administration prolongee de fortes doses de ce sel. *Bull. et. Mem. Soc. Med. d. Hop. d. Paris.*, 1922, No. iv, 214-220.

[2] Singer, R., Das Kalzium in der Herztherapie. *Therap. Halbmonatsh.*, 1921, xxxv, 758.

[3] Loewi, O., Über den Zusammenhang zwischen Digitalis—und Kalziumwirkung. *Arch. exp. Path. u. Pharmakol.*, 1917, lxxxii, 131, and 1918, lxxxiii, 366.

TABLE I.

Patient	Calcium chloride				No. of observations on each patient.
	With digitalis		Without digitalis		
	Diuresis	No diuresis	Diuresis	No diuresis	
F.	3*	0	4	0	7
C.	2	0	3	0	5
A.	1	1	0	0	2
K.	1	0	0	0	1
B.	0	0	1	0	1
Total	7	1	8	0	16

*Refers to number of separate observations.

but in the three remaining cases the diuresis was not sufficient to make any impression on the degree of the edema.

Before giving calcium chloride intravenously to patients we took the precaution of making preliminary observations on dogs with the view to learning the dose with which toxic effects on the heart are induced.

We have injected 0.5 to 1.1 gm. of calcium chloride (10 per cent solution) intravenously in dogs without the appearance in electrocardiograms of ventricular premature contractions or ventricular standstill. In one dog we injected intravenously at the same time 0.5 gm. calcium chloride and 30 per cent of the calculated lethal dose of digitalis. About 40 minutes after the injection numerous ventricular premature contractions appeared and were still present 1½ hours after the injection, but had disappeared the next day. One week later the experiment was repeated with the same results. One week after this a smaller dose (25 per cent of the calculated lethal dose) of the same tincture was given with the same amount of calcium chloride but without causing an irregularity. A second dog was given 25 per cent of the calculated lethal dose of the tincture of digitalis intravenously without causing an irregularity. Two hours later 0.5 gm. of calcium chloride was given intravenously and ventricular premature contractions failed to develop. One week later when 30 per cent of the calculated lethal dose was injected, followed by the same amount of calcium chloride, a slight extrasystolic irregularity developed. It appears then that about 30 per cent of the calculated lethal dose of digitalis is critical when combined with calcium.

On the basis of this experience we have given 0.1 to 0.2 gm. of calcium chloride each day for two to six days to three patients on six occasions. It was found that no definite change in the urinary output took place, although in one patient (F) it had been found repeatedly that when calcium chloride was given by mouth diuresis occurred. At this time the difference between the effects obtained by administration by mouth and by the intravenous method is not discussed but is reserved for a future occasion. A short time after calcium chloride was injected the radial pulse usually became more forcible. Calcium chloride in these doses did not produce any change in the form of the electrocardiogram. In the patient (F) mentioned above, 0.5 gm. of digitalis was given by mouth on the day following the last intravenous calcium chloride injection, and then the output increased and remained elevated until the patient was free of edema. With this the pulse rate slowed and the electrocardiogram showed changes in the T-wave. Before this the patient had many times been given much larger doses of digitalis, but these had never been followed by a diuretic effect which continued long enough to free the patient of edema as has followed this small amount (0.5 gm.) of digitalis when combined with calcium.

It appears then that diuresis can be obtained by the administration of calcium chloride by mouth although the amount is small. It appears also that when combined with digitalis the amount of this drug required to bring on diuresis may be reduced.

191 (2423)

A method for obtaining samples of mixed venous blood in intact dogs.

By HAROLD J. STEWART.*

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

In order to estimate the oxygen and carbon dioxide in mixed venous blood in intact dogs two methods are in use: (1) a tonometer method in which alveolar air and pulmonary venous blood are allowed to come into equilibrium; the air is then analyzed. (2) A method of direct puncture of the right auricle or ventricle in order to obtain venous blood. A third method is possible and is now described.

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*Introduced by Alfred E. Cohn.

We devised a cannula which can be inserted into the right ventricle through the right external jugular vein. Through this samples of mixed venous blood can be withdrawn from the right heart. The cannula is a hollow metal tube 2.5 mm. in diameter and 30 cm. long, having an obturator which is bullet shaped closing its distal end. The obturator can be withdrawn and the proximal opening closed by a small stopcock. Below the stopcock is an outlet to which a piece of rubber tubing is attached Before use the air in the cannula is replaced by sterile albolene.

The operation is carried out aseptically. The skin and subcutaneous tissue along the line of incision are anesthetized with 1 to 2 per cent novocaine. The right external jugular vein can now be exposed by blunt dissection without discomfort to the dog. The distal end of the vein is ligated. A small opening is made into the vein below the ligature and the cannula inserted and tied in place. The cannula with the obturator in place is pushed into the innominate vein, superior vena cava, and then into the right heart. At first the operations were carried out under the guidance of the fluoroscope, but this was soon found to be unnecessary because by turning the dog slightly on his left side the line along the external jugular vein to the heart becomes practically straight and the cannula can be made to enter the heart easily. When in place the cannula can be felt to move with each systole. When the obturator is withdrawn and the stopcock closed blood flows from the outlet. The blood is collected by placing the rubber tubing under albolene or by using an air free syringe attached to the rubber tubing.

After a satisfactory sample has been obtained, the obturator is returned, the cannula removed, and the vein ligated below the point of entry. The skin is closed with a silk suture and protected with a dry dressing. The procedure requires less than a half hour. By inserting the cannula as high in the neck as possible the first time, the operation can be repeated many times, each time inserting the cannula below the point of the previous ligation. The vein remains patent below, but in time is obliterated above the point of ligation. When it is no longer possible to use the external jugular vein, the internal jugular vein can be used in a similar manner. The left external jugular vein is more difficult to use because of the angle at which the vein enters the innominate vein.

Samples of blood from the left heart may be obtained from the right carotid artery at the time the external jugular vein is exposed or may be obtained more easily from a femoral artery. The femoral artery in the dog is quite superficial and an arterial puncture is easily and quickly done without any discomfort and with safety if pressure is applied over the artery for a few minutes after the puncture.

192 (2424)

The rôle of the liver in pancreatic secretion.

By N. H. PLUMMER, H. J. DEUEL, JR., and G. R. COWGILL.

[*From the Department of Physiology, Cornell University Medical College, New York City, and the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

Secretin, when injected into dogs under amytal or ether anesthesia, proved far less effective in exciting the pancreatic flow when introduced by the portal vein than when administered by way of the femoral vein, thus confirming an earlier observation.[1] It was likewise found that with extremely potent secretin solutions, the difference in effect by the portal and systemic routes is much less marked than with weaker preparations.

In view of the fact that the secretin when injected by the portal system must first pass through the liver, it may be suggested that this organ in some way is responsible for the different behavior noted above. In order to further investigate this, secretin solutions were injected at a slow rate over a long period of time by each of these routes. When injected at the rate of one-half cubic centimeter per minute by the femoral vein, an increased secretion of the pancreatic juice was noted in the first five minute period and a constant maximum response was reached in the third five minute period. On injection of the same secretin preparation at the same rate by the portal vein, no effect could be noted for thirty minutes and the constant maximum flow of the pancreatic juice was not obtained for forty minutes; however, if a second injection of secretin solution at the slow rate was made by the portal immediately following the cessation of the flow as the result of the previous slow injection, an effect on pancreatic secretion was now noted in the second five minute interval and the constant maximum flow was obtained within twenty minutes. This result would indicate that the liver is able to absorb a considerable amount of secretin; when it becomes saturated by an injection of a relatively large amount of this substance, this power of absorption is lost and the effect obtained with secretin injections by the portal more nearly simulates that obtained by the administration by a systemic vein.

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[1] Deuel, H. J., Jr., and Cowgill, G. R., presented at the XIth International Physiological Congress, Edinburgh, 1923.

A N HCl extract of fresh liver prepared by the methods usually followed in making secretin, gave a potent secretin. That the response of the pancreas following the administration of this fresh liver extract was not due to a vasodilator, is shown by the fact that the HCl extract of liver previously dehydrated with alcohol—a procedure which will remove the vasodilator—still provoked a strong effect on pancreatic secretion.

Just what rôle the liver ordinarily plays in pancreatic secretion can not be stated. That it does not store the secretin in order to prolong the activity of the pancreas seems certain in view of the fact that the response of the pancreas is always of shorter duration when the secretin preparation is injected by the portal than that observed when the same injection is made by the femoral. Obviously this organ must destroy secretin (or eliminate the part stored at such a rate that it is impotent) since the normal liver is found to be desaturated.

193 (2425)

Further studies on the effect of bacillus acidophilus therapy on indican excretion.

By LILLIAN SEGAL KOPELOFF.*

[*From the Department of Biological Chemistry, New York State Psychiatric Institute, Ward's Island, New York City.*]

The beneficial effects obtained with *B. acidophilus* have raised some interesting problems. One of the most recent to receive attention is the influence of *B. acidophilus* ingestion on intestinal putrefaction, particularly as measured by the excretion of indican in the urine. Kopeloff[1] has demonstrated the therapeutic value of *B. acidophilus* milk in correcting constipation in psychiatric patients. The present investigation is concerned with the indican excretion of these patients as compared with that of a similar group which had received no bacillary treatment. It was undertaken because of the frequent assertions in the literature regarding the intimate and causal relationship between indicanuria and mental disease; and with the hope that alleviation of constipation might be found to affect the indican excretion and some light be thereby thrown on the connection between the indican excretion and the mental condition.

About 500 urinalyses were made on 48 psychotic patients of whom 25 were control cases receiving no treatment. Of the controls 4 were extremely constipated, one had diarrhea, and the remaining 20 had good daily defecations. Twenty-four hour specimens of urine were collected and the following determinations made: volume, specific gravity, color, acidity, and indican according to the method of Myers and Fine. A record was kept of the kind and amount of food consumed by each patient daily, and the number and quality of bowel movements. The fecal flora were determined weekly, both plate and microscopic counts. Accepting 5 to 20 mg. daily excretion as the normal limits there were only 13 instances of abnormal excretion in the 500 analyses. Curiously enough six of these occurred in patients having good daily defecations, while six occurred in constipated subjects. The highest value, 46.5 mg., occurred in a patient with diarrhea.

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* Introduced by Nicholas Kopeloff.

[1] Kopeloff, N., *Arch. Int. Med.*, 1924, xxxiii, 47.

There were only 25 instances of an excretion of 15 to 20 mg.; 11 of these were of constipated subjects, 14 of the patients who had good daily defecations. The remaining determinations were all under 15 mg. and about 300 of these were less than 5 mg.

The intestinal flora of many of these patients was transformed from the putrefactive to the aciduric type and frequently the fecal counts showed a transplantation of 95 per cent *B. acidophilus*. The indican excretion continued variable, however, no correlation being observed between that and the intestinal flora.

The titratable acidity, determined by Folin's method, was found to vary with the *B. acidophilus* preparations. Various preparations were used as previously described by Kopeloff[2] There was a distinct rise in the acidity when *B. acidophilus* milk was taken, and an even more conspicuous increase when lactose was added. This indicates the need of caution in feeding large amounts to individuals who may already have a tendency towards acidosis.

194 **(2426)**

Immunity results obtained in school children with diphtheria toxoid (modified toxin) and with 1/10 L+ mixtures of toxin-antitoxin.

By ABRAHAM ZINGHER and WM. H. PARK.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

Excellent immunity results obtained with 3 L+ mixtures of toxin-antitoxin, which were originally underneutralized but had become on standing neutral for the guinea pig, indicated that the toxoid into which the free or slightly bound toxin had been changed was effective as an immunizing agent. Dissociation of the mixture into toxin and antitoxin is probably not of much importance as a factor in active immunization since mixtures that are overneutralized or just neutral when freshly prepared do not give good immunity results. Three doses of a freshly diluted toxin were found to immunize only 33 to 41 per cent of susceptible children, while three doses for a dilution of an old, deteriorated toxin, of which 1.0 cc. caused paralysis in the guinea pig, immunized 70 per cent of children. The value of toxoid for immunization purposes has been confirmed by using modified diphtheria toxin, treated by the addition of 0.1 per cent formalin according to the suggestion of Glenny and Hopkins.

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[2] Kopeloff, N., *J. Am. Med. Assn.*, 1923, lxxx, 602.

In four schools in which a preparation of toxoid has been used the following very significant results were obtained at the end of three months:

Public Schools	Toxoid			Children retested		
	Dilution	Per cent in dilution	Amount injected	Total	Schick negative	Per cent negative
103 Man.	1:20	5.0	1 cc.	112	94	84.0
83 Man.	1:14	7.5	1 cc.	67	66	98.5
19 Man.	1:14	7.5	1 cc.	122	120	98.3
14 Man.	1:14	7.5	1 cc.	100	98	98.0

The toxoid was made from a diphtheria toxin which at the time of preparation had an M.L.D. of 0.001 cc. Two years later the M.L.D. was 0.01 cc. and the L+ 0.32 cc. 0.1 per cent formalin was added (1 cc. commercial formalin to 1000 cc. of toxin) and the preparation kept in the thermostat for five weeks. At the end of this time the M.L.D. had dropped to 1.5 cc. and the L+ to 3.5 cc. The toxoid was diluted 1 part in 20 parts of saline (5 per cent) for P. S. No. 103 and 1 in 14 (7.5 per cent) for P. S. Nos. 83, 19, and 14. In other schools that are being retested now the dilution of the toxoid was 1 in 10 (10 per cent). *It is interesting to note that toxoid acted as a very efficient immunizing agent even when the free toxin in each dose was less than* 1/30 *M.L.D.* The toxoid was prepared by Dr. Banzhaf of the Research Laboratory, who has obtained similar immunity results by the injection of guinea pigs with this preparation.

The local reactions after the intramuscular injections of the diluted toxoid were only slight. As we would expect from the character of the preparation there was very little free toxin action and most of the local reaction after the injections was due to the protein content of the diluted toxoid. This was verified by noting the slightly more marked local effects in positive-combined Schick reactors. Only a few children showed slight constitutional symptoms. The local reactions were no more marked than after the use of the new 1/10 L+ mixtures. The immunity results were very good and compared favorably with those noted after the use of 1/10 L+ mixtures..

1/10 L+ mixtures of toxin-antitoxin have given successful immunity results in from 80 to 95 per cent of injected school children. *At the Schick retest many of these children show a moderate to marked pseudo-reaction.* This indicates the necessity of always making a control test when the Schick retest is made to check up on the immunity results. Mixtures containing 1/10 L+ lose some of their immunizing efficiency on standing at icebox temperature for several months. In judging the immunity results after injections of toxin-antitoxin we have to consider not only the special mixture, but also the individual group of children treated and the period of time elapsed after the injections.

The time factor in the Schick retest is especially important. More than 50 per cent of those who were found to be still susceptible at the end of three to six months gave a negative Schick reaction when retested one year later without having received further injections of toxin-antitoxin.

195 **(2427)**

The significance of the pseudoreaction in the Dick test and methods used for its identification.

By ABRAHAM ZINGHER.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

Pseudoreactions due to the autolyzed streptococcus protein and other protein constituents of the toxin broth test fluid have an important bearing in the interpretation of the Dick reaction. Of 589 children tested in one of the public schools 459 were found to be immune. Of these, 190 or 41.4 per cent gave a pseudoreaction. Of the 130 susceptible children, 45 or 34.6 per cent gave a combined positive and pseudo reaction. Of another group of 950 children, who received the Dick test in two institutions, 686 were found to be immune. Of these, 232 or 33.8 per cent gave a pseudoreaction. Of the 264 non-immunes, 90 or 34.0 per cent gave a combined reaction. In children under 5 years the percentage of negative pseudo and positive combined reactions is much less and is closely analogous to the similar small percentages of pseudo reactions found with the Schick test in this age group.

To make the readings of the Dick reactions more accurate and avoid the confusion that is very likely to arise between positive and negative pseudoreactions it is important to use a control test, which will show with a great deal of accuracy the difference between the four different reactions described before this society at its March meeting in connection with the Dick test: the positive, negative, negative pseudo and positive combined. The control test is thus closely analogous to the one used with the Schick test and serves the same purpose. It is even more important, however, to use a control with the Dick test than it is with the Schick test where it is strongly indicated in older children and adults. As the positive reactions appear within 24 hours the time element used by some to separate the positive and negative pseudoreactions in the Schick test is practically of little or no value in differentiating the reactions noted with the Dick test.

For the control three different solutions can be used: (1) A dilution of the toxin which contains 25 per cent of convalescent serum or plasma, obtained from three weeks' scarlet fever convalescents. To make the dilution add 1 cc. of 1:10 dilution of the toxin to 75 cc. saline (containing 0.25 per cent phenol) and 25 cc. of convalescent serum or plasma. This gives an ultimate dilution of the toxin 1:1000 which would be the same as that used for the Dick test. If the ultimate dilution of the toxin should be 1:500, add 2 cc., etc.

(2) A dilution of the toxin which contains 25 per cent of normal serum or plasma, obtained from individuals who give a negative Dick test. The dilution is made in the same manner as with convalescent serum.

(3) Streptococcus scarlet fever toxin which has been heated in a water bath at boiling temperature for one hour. The toxin that gives rise to the positive reactions is destroyed by the heat, while the reacting proteins that cause the pseudoreactions are not affected. It is best to heat the toxin in a primary dilution of 1:100, the final dilution being made as required. The final dilution, however, as used for the Dick test, can also be heated for one hour and then used without further dilution as a control.

The heated scarlet fever streptococcus toxin may be ultimately used as a control as it can be more conveniently prepared. However the following results are rather significant: In a group of 200 children who received the Dick test and two controls (heated toxin and toxin neutralized with 25 per cent mixed convalescent plasma) 52 or 26.0 per cent gave positive Dick reactions, 40 or 20.0 per cent showed a slight reaction with the heated control and no reaction with the neutralized control and 5 or 2.5 per cent gave combined reactions, in which both controls appeared, but were much less marked than the Dick reaction. Of the immune children, 80 or 40.0 per cent gave a negative Dick reaction and negative controls and 23 or 12.5 per cent gave negative-pseudo reactions, in which the Dick test and the two controls were exactly similar. *It is interesting to note that in this group of 200 children there were 40 on whom the neutralized convalescent plasma toxin acted as a more perfect control than the heated toxin.*

All dilutions of the scarlet fever toxin should be made with normal saline (0.85 per cent) containing 0.25 per cent phenol if they are to be kept for a few days.

The final dilution (1:1000) of the scarlet fever streptococcus toxin is more stable than the final dilution of diphtheria toxin for the Schick test. The final dilution can be kept and used for two weeks and probably longer without noticing any appreciable diminution in its toxic strength.

196 (**2428**)

The irritating local effect of human blood serum or plasma containing chyle obtained soon after meals.

By ABRAHAM ZINGHER.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

In carrying on work with the intradermal blanching test in scarlet fever rashes we noted that serum or plasma obtained from human beings soon after meals and containing a considerable amount of chylous fluid produced a distinct local irritating effect on the human skin. The irritating action showed itself by a local inflammatory area that appeared on the following day. In some of the tests there was a minute superficial postule, which contained polynuclear leucocytes, but no bacteria. Blood serum or plasma taken from the same individuals before meals and appearing quite clear did not show this irritating local action. Studies are being carried out at the present time to see whether the same holds true for bleeding from animals taken before and after meals.

This factor of the irritating quality of chyle-containing plasma and serum will have to be taken into consideration when blood is withdrawn from animals that will be used for injections of human beings. The increasing application of human sera obtained from convalescent and normal individuals in the prophylaxis and treatment of various infectious diseases like measles, scarlet fever and poliomyelitis indicates its importance. In treating cases of scarlet fever with intramuscular injections of whole blood, as we recommended a few years ago, we noted in two individuals fairly marked local reactions at the sites of injection. A few of the other patients showed slight or moderate reactions. Although our attention was not drawn to it at the time these patients were treated it is probable that the local irritating effect of the homologous blood was caused by the same factor.

It is possible also that some of the unexplained reactions following blood transfusions carried out by expert operators with perfect technique will be cleared up if note is taken of the food intake of the blood donors. It would follow that such donors should not partake of heavy meals before giving blood.

The removal of blood for therapeutic purposes from convalescent patients and normal individuals should, therefore, be carried out before meals. It is interesting to note in this connection that some laboratories have been in the habit of bleeding animals furnishing antitoxic and various bacterial antisera after fasting them for twenty-four hours.

197 (2429)

Observations on human isoagglutinins.

By KARL LANDSTEINER and DAN H. WITT.

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It has been known for a long time that there are certain exceptions to the common blood grouping. This was pointed out repeatedly by one of the writers[1] as well as by others.[2]

It did not seem worth while to describe in detail some observations which we have made along this line since, at the present time, there is no practicable way of comparing such disconnected findings with similar ones of other workers and properly classifying them.

In our studies we have found instances of weak agglutination of a serum type I with another blood of the same type and recorded similar reactions with specimens of types II and III.

The reactions were chiefly observed at a temperature of about 15° C. or less. At higher temperatures they became gradually weaker and disappeared. Several times the clumps contained rouleaux.

The observation that at lower temperature atypical agglutinin reactions may occur between human bloods and sera of different individuals has already been described by Bialosuknia and L. Hirszfeld.[3] While the conclusion of these authors that the reactions are different from isoagglutination is questionable, they certainly gave a description of the fact. Similar phenomena were described in detail by Guthrie and Pessel.[4] Although it is possible that an agglutination of this kind would not occur at body temperature yet the slight difference in the constitution of the blood might be sufficient to produce undesirable effects. Regarding the practical application, the observations suggest the use of direct cross tests before transfusion (in addition to typing); furthermore, it seems advisable to make these tests at low temperatures since in that way slight reactions may appear. This deduction has previously been made by Guthrie and Pessel.

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[1] Landsteiner, K., *Wiener klin. Wochenschr.*, 1901, xlvi, 1132. Landsteiner, K., *Oppenheimer Handb. d. Biochemie*, 1909, ii, 414. Landsteiner, K., *Zentr. Gynäkologie*, 1921, xlv, No. 19.

[2] V. Dungern, E., and Hirschfeld, L., *Z. Immunitatsf*, 1911, viii, 527. Ottenberg, R., *J. Exp. Med.*, 1911, xiii, 425. Unger, L. J., *J. Am. Med. Assn.*, 1921, lxxvi, 9. Guthrie, C. G., and Huck, J. G., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 37. Hooker, S. B., and Anderson, L., *Am. J. Immunol.*, 1921, vi, 419.

[3] Bialosuknia, W., and Hirszfeld, L., *Przegladu Epidemjologicznego*, 1921, i, 437.

[4] Guthrie, C. G., and Pessel, J. F., *Johns Hopkins Hosp. Bull.*, 1924, xxxv, 33.

Difficulty may be experienced in distinguishing isoagglutination at low temperatures from autoagglutination since the latter is favored by decreasing temperature.[5] To control this point, tests should be made using the cells of the individual from which the serum is taken and cells of several other individuals, in case an irregular reaction is observed.

The finding of "atypical" isoagglutinin reactions does not alter the fact that the division of human blood in four groups is a fair approximation but there is at least one addition to the established facts concerning isoagglutination[5, 6] which can already be classified and easily detected.

V. Dungern and Hirschfeld[7] found that after absorption of a serum, type III[8], with a certain blood II, agglutinins remained for a number of blood samples II and not for others of the same group. To explain the phenomenon, one may assume the existence of a special agglutinable substance in those which reacted. Analogous observations were made in an elaborate study by Guthrie and Huck.[9] They designated the agglutinable substance of group II detected in this way and by a special serum, with the letter "C".

Coca and Klein[10] discovered a similar agglutinable element by means of a serum, group I, absorbed by a certain blood II. Since they found their factor, "x," present in about 78 per cent of bloods belonging to group II while Guthrie and Huck had found not more than 25 per cent, they concluded that the two agglutinogens are probably not identical. In a recent communication, however, Guthrie and Pessel[11] state that the frequency of the finding is higher than previously considered.

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[5] Landsteiner, K., *Münch. med. Wochenschr.*, 1903, 1812.

[6] von Decastello, A., and Sturli, A., *Münch. med. Wochenschr.*, 1902, 1090.

[7] V. Dungern, E., and Hirschfeld, L., *Z. Immunitatsf.*, 1911, viii, 527.

[8] According to the American Nomenclature, *J. Am. Med. Assn.*, 1921, lxxvi, 130.

[9] Guthrie, C. G., and Huck, J. G., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 37.

[10] Coca, A. F., and Klein, H., *J. Immunol.*, 1923, viii, 477.

We have found an agglutinable factor in group II blood other than the common "A" by making tests with a serum of a patient, Barnett. The corpuscles of this individual were agglutinated by all sera I, II, III and, therefore, had to be put in group IV. The serum, on the other hand, agglutinated a number of bloods of group II and one of group IV. They behaved thus like certain absorbed sera I and III in the experiments of v. Dungern and Hirschfeld, Guthrie and Huck, Coca and Klein.

The reactions in our tests were not very strong but quite distinct. They disappeared almost entirely at 37° C. (The tests were made at 20° by mixing equal parts of serum, 2.5 per cent blood and saline.)

Of 21 bloods, type II, tested, 16, *i. e.*, about 76 per cent, gave positive reactions, 5 negative reactions, with serum Barnett in agreement with the figure found by Coca and Klein.

We made parallel tests with a number of bloods, type II, using the serum Barnett and serum A. F. C. (I) absorbed with blood Levine (II). This material was kindly furnished us by Dr. Coca. There was conformity of the reactions in all 15 cases as indicated by the following table containing a part of the tests.

	1	2	3	4	5	6	7	8	9
Barnett	+	±	±	±	±	+	0	+	0
A.F.C. absorbed with Levine	+	±	±	tr.	tr.	±	0	±	0

The corpuscles of blood Barnett were not affected by serum of A. F. C. absorbed with corpuscles Levine. They, evidently, do not contain the agglutinable element on which the serum acts. Another blood IV tested by us does contain this element. We have found an agglutinin of a similar kind as that described in another of several sera, group IV, tested. Thus the occurrence is apparently not unique.

According to our results group IV contains two sub-groups. In one there is an agglutinin in the serum, in the other, none.

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[11] Guthrie, C. G., and Pessel, J. F., *Johns Hopkins Hosp. Bull.*, 1924, xxxv, 23.

They may be designated as follows:

	IV (1)	IV (2)
Serum	—	c.
Corpuscle	A.B.C.	A.B.

The agglutinable factors "c" or "x" in cells of group II and IV found by previous workers and in our cases are probably identical.

198 (2430)

Filter-passing bacteria in the nasal passages of animals.

By PETER K. OLITSKY and DAVID T. SMITH.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

Anaerobic cultures of the filtered nasopharyngeal secretions from man reveal a varied bacterial flora. There have thus far been described, *Bacterium pneumosintes,*[1] obtained from fresh cases of typical epidemic influenza, and three distinct groups[2] of Gram-negative, filterable, anaerobes, derived from patients with influenza, obscure upper respiratory affections, or common colds, as well as from persons supposedly healthy. To the latter three groups may be added another related species which will be described later and which has been isolated from a number of cases of infectious common cold.

The object of the present study was to determine whether, and to what extent, this class of microorganisms is distributed among laboratory animals. Accordingly, the filtered materials obtained from the nasal mucosa of 10 monkeys, 7 horses, 17 dogs, 5 cats, 20 rabbits, 20 guinea pigs, and 20 rats were cultured by the same methods which had yielded growth of the different species of bacteria from human secretions.

Suspensions in one per cent dextrose Ringer's solution were prepared of the nasal mucosa, dissected or curetted away from the underlying bone of recently killed animals, or of the nasal secretions collected on cotton swabs of living ones. The suspensions were filtered through Berkefeld "V" candles, impervious to Pfeiffer's bacilli or *Bacillus prodigiosus,* at 650 mm. Hg negative pressure. The culture media[2] used were the Smith-Noguchi fluid medium and rabbit blood agar plates, and the cultures were incubated for 7 days under anaerobic conditions in Brown's jar—a modification of the McIntosh and Fildes apparatus.

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[1] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 713.

In no instance, thus far, have any filter-passing microorganisms been cultivated from this material by the methods employed.[3]

199 (2431)

Ovogenesis during sexual maturity as elucidated by experimental methods.[1]

By GEORGE N. PAPANICOLAOU.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

It has been repeatedly claimed that ovogenesis continues during sexual maturity but the evidence so far has not been considered entirely conclusive. On this account the more popular view among morphologists today is that ovogenesis in mammals ceases before puberty. The primary ovocytes contained in the mature ovary are not thought to be newly formed but simply stored there for later use.

The chief reason that a demonstration of post-pubertal ovogenesis has been so difficult is on account of the fact that the process in normal mature ovaries is much retarded and easily overlooked.

Recently my attention to this problem has been attracted by studying a series of ovaries from guinea pigs, in which the thyroids have been treated with radium emanation. Some of these ovaries had their germinal epithelium so much activated that all stages in post-pubertal ovogenesis could be seen and demonstrated almost as well as in young immature ovaries. By concentrating attention on this point I was later able to observe the activation of the germinal epithelium by other factors. Every factor that increases the congestion and the blood supply in the ovary seems to produce such an activation. Injections of cystic fluid and of other glandular extracts may produce this effect.

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[2] Olitsky, P. K., *J. Exp. Med.*, 1922, xxxvi, 501; Olitsky, P. K., and McCartney, J. E., *J. Exp. Med.*, 1923, xxxviii, 427.

[3] In the case of two rabbits both methods yielded growth of a minute, gas-producing microorganism, the *Staphylococcus parvulus,* wholly unrelated to any of the bacilloid, bacillary, or vibrio-like bacteria of human origin. These cocci were filterable, under the condition of the experiment, only in the early generations.

[1] This work has been supported by the Committee for Study of the Sex Problem of the National Research Council.

This experimental stimulation of post-pubertal ovogenesis led me to study a large number of ovaries from normal guinea pigs at different life periods as well as from embryos and from the pregnant female. From this study a number of points of importance have been derived.

There is a continuous process of ovogenesis from the time of the gonadal differentiation in the embryos up to the time of cessation of sexual activity in the older female. This process decreases in activity with progressive age and almost ceases in the old female.

Ovogenesis is not entirely uniform but varies in intensity at different times. All factors that increase genital activity tend to increase this process. Favorable nutritive or seasonal conditions, estrous activities or any factor increasing the blood supply in the genital organs act in this direction. Seasonal variations and cyclic changes tend to give to it a periodical expression.

Other factors act in an inhibitory way. Unfavorable nutritive or seasonal conditions, diestrous inactivity and especially increase in developmental competition within the ovary act in an inhibitory manner. The appearance and presence of active *corpora lutea* within the ovary gives the most severe setback to the ovogenetic process. For this reason during pregnancy or during diestrum when the ovaries contain active *corpora lutea* the ovogenetic process is lowered in activity. There is, however, a slight new production of primary ovocytes continued during diestrum or pregnancy which increases with the decrease of function and activity of the *corpora lutea*—that is, during the latter part of these periods.

Therefore, at all times, even during diestrum and during pregnancy, a number of new primary ovocytes are formed and a number of these begin to develop within the ovary. There is evidence that every primary follicle must grow or die. Long persistence of any follicles of any size within the ovary seems to be quite impossible. Any theory based on the assumption that primary ovocytes persist for long periods of time or throughout the life of the organism is untenable.

The existence of a continuous ovogenetic growth within the ovary is a very fortunate arrangement, tending to supply fresh developing ova within the ovary at all times. The actual rhythm is expressed more clearly in the large follicles and the *corpora lutea,* which are periodically destroyed and reformed.

The germinal epithelium of the ovary gives rise not only to primary ovocytes, but also to follicular cells, and interstitial groups. In young ovaries there is an excess of primary ovocytes and interstitial groups, while in mature ovaries there is an excess in production of follicles and luteal cells. The follicular cells are steadily formed and migrate into the stroma of the ovary in order to supply the growing follicles with an additional number of such cells. The primary follicles do not depend on the small number of follicular cells around them nor on their slow multiplication to provide the material for their growth, but they are steadily acquiring new follicular cells and later on luteal cells from new cells derived from the ovarian epithelium.

This migration is also participated in by the primary ovocytes, which move into the interior of the ovary and degenerate, if they have not the chance to develop further.

Only a few of the primary follicles develop further. Most of them degenerate in the primary ovocyte stage. This is especially the case with the large groups of ovocytes, which are sometimes accumulated together and covered by a single layer of follicular cells. Their poor follicular supply does not allow them to compete with other more isolated and better supplied ovocytes.

The ovocyte groups mainly appear after an active period of ovogenesis and are produced by a group-like differentiation of germinal epithelium cells. This may happen only under very favorable conditions; otherwise such groups differentiate into interstitial clumps. It seems to me that the interstitial groups are nothing more than abortive groups of primordial ovocytes showing signs of progressive fatty degeneration. One has to draw a line between the ovogenetic activity and the relative differentiatiation of the epithelial cells. At times there is a rich growth of ovocytes, while at others a higher growth of follicular and luteal cells or a richer production of interstitial groups.

The activation of the epithelium is not uniform over the entire ovary, but it is rather localized in different areas. In some places, probably on account of a better blood supply and better nourishment, the epithelial cells, which are to be considered as resting ovogonia, begin to grow and their nuclei display some of the morphological changes that characterize the growth of spermatogonia into primary spermatocytes. In other places the epithelium may be found entirely inactive.

Mitotic figures can be seen in the epithelium but they are not as common as one might expect if every epithelial cell were to divide before giving rise to a primary ovocyte. It seems, therefore, that the ovogonia of the germinal epithelium may grow into an ovocyte without a previous mitotic division.

200 (2432)

The appearance of specific antibodies in the serum of rabbits by intratracheal and intravenous injections of living tubercle bacilli.

By B. M. FRIED and E. E. PROCTOR.*

[*From the Department of Pathology, Harvard Medical School, and the Pathological Laboratory of the Boston Sanatorium, Boston, Mass.*]

The production of antibodies in animals (rabbits) by the intratracheal injection of bacterial antigens other than tubercle bacilli is considered as practically identical to the intravenous injection.

The importance of the introduction of antigens directly in the lungs has received a particular importance in relation to recent investigations concerning "autonomy of organs" (Besredka[1]) in infection and immunity. It is of interest to note Besredka's experiment in vaccination against diphtheria. A repeated intratracheal injection of killed diphtheria bacilli will protect a guinea pig against a subcutaneous injection of living Klebs Loeffler bacilli, while the control animal vaccinated subcutaneously will die from the same dose of the living bacilli.

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* Introduced by S. B. Wolbach.

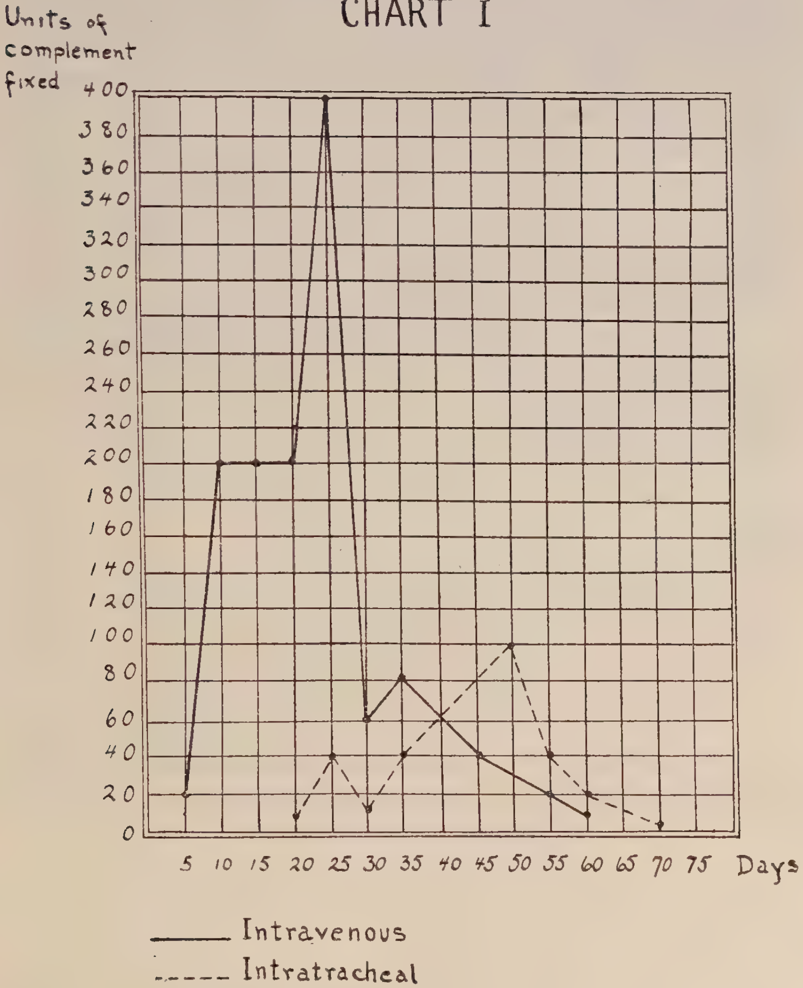
[1] Besredka, A., *Ann. de l' Institut Pasteur*, 1919, 1920, 1921.

Our experiment is a comparative study of the production of specific antibodies by the intratracheal and intravenous injection of living tubercle bacilli in rabbits (human and bovine strains) and in guinea pigs. A study is also being made of the appearance of the intradermal tuberculin test in guinea pigs inoculated with living tubercle bacilli intravenously and intratracheally.

This preliminary report deals only with the results obtained by the injection of a human strain of tubercle bacilli in rabbits.

Method. The intratracheal injection was performed through

CHART I



the lower rings of the exposed trachea, care being taken to avoid local contamination of attaching the syringe only when the needle was inserted in the trachea. The customary marginal vein of the ear was utilized for intravenous injection.

Antigen. A six-weeks old human strain of living tubercle bacilli (H. 60. Allen K. Krause) grown on agar glycerine was finely emulsified and one milligram of the tubercle bacilli in one cc. of normal salt solution was injected. The animals were bled at regular intervals and the complement fixation test performed with the inactivated serum in the presence of Besredka's tuberculous antigen[2] using 1/6 of its anticomplementary unit, two units of complement and four units of amboceptor. The antibodies were titrated with a fixed amount of complement, amboceptor, antigen and decreasing quantities of serum.

Experiment. Five rabbits (R1-R5) were inoculated intravenously and another five (R6-R10) intratracheally, each animal receiving a single dose as stated above. The average weight of each rabbit was 2000 to 2100 gm.

It will be noted from the table that:

1. By intravenous injection, antibodies detectable by the complement fixation reaction were already present in the serum of the animals at the end of the first week (5th to 7th day) after inoculation. After a rapid rise the strength of the test was maintained at the same level for about two weeks, followed by a second rise, reaching its maximum at the end of the first month (400 units) and from that time it grew weaker and weaker.
2. By intratracheal injection antibodies first appeared only at the end of the third week after inoculation rising to its maximum at the end of the sixth week (after inoculation) and from that time it waned gradually in strength.

Conclusion. The intravenous route in the production of specific antibodies by the injection of a human strain of living tubercle bacilli is superior to the intratracheal. In the former the antibodies appear earlier in the serum and are greater in number.

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[2] Fried, B. M., Besredka's tuberculosis antigen and the complement fixation reaction, *Am. Rev. Tuberculosis* (in press).

201 (2433)

The fundamental erythrocytopoietic stimulus.

By H. E. JORDAN and C. C. SPEIDEL.

[*From the Laboratory of Histology and Embryology, University of Virginia, Charlottesville, Va.*]

The problem of determining the factors involved in the stimulation and regulation of erythrocyte production has been attacked both from the morphological and physiological point of view. Following detailed studies on both normal and experimental animals some hematologists, *e. g.,* Maximow[1] and Danchakoff[2], have concluded that the differential factor responsible for the development of an erythrocyte from a lymphoid hemoblast is intravascular location. However, the specific feature of intravascular location that stimulates erythrocytopoiesis, whether the mechanical condition of envelopment by endothelial wall, or certain chemical substances in the blood, remains unidentified.

In regard to the alleged instances of extravascular erythrocytopoiesis among birds and mammals, Danchakoff is inclined to believe that many, if not all, of these should be interpreted as cases in which the primary stimulus is nevertheless applied intravascularly, the hemoblasts thus receiving an initial and irreversible bias in the direction of an erythrocytogenic differentiation which is later completed extravascularly. In this connection it may be noted that Doan,[3, 4] and Cunningham and Doan[5] have recently pointed out that the capillary network in the bone marrow of birds and mammals is much more extensive than is commonly believed, and suggest that much of the supposed extravascular erythrocyte formation may in reality be intravascular. Latta[6], however, claims to find true primary extravascular erythrocytopoiesis in the intestinal wall of the rabbit. Accordingly, he substitutes for "intravascular conditions", the condition of "favorable relation to transudations from the blood plasma", and he believes that there is something in the balance of chemical substances in the blood plasma which determines erythrocytopoiesis. The evidence from histological studies indicates that close relation to the blood stream, and very slow blood flow, are chief conditions necessary for red-cell differentiation.

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[1] Maximow, A., *Arch. mikr. Anat.,* 1909, lxiii, 444.

[2] Danchakoff, V., *Am. J. Anat.,* 1918, xxiv, 1.

[3] Doan, C. A., *Johns Hopk. Hosp. Bull.,* 1922, xxxiii, 222.

[4] Doan, C. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 260.

[5] Cunningham, R. S., and Doan, C. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 262.

[6] Latta, J. S., *Am. J. Anat.,* 1921, xxix, 159.

Among the results obtained by various investigators studying the problem experimentally from the functional viewpoint may be mentioned the following: Carnot and Deflandre[7] have suggested the presence of a "hemopoietin" and a "hemolysin" in normal blood in a certain balance. They believe that an increase in erythrocyte formation is brought about by any stimulus that will cause the hemopoietin to predominate, and conversely that any stimulus causing the hemolysin to predominate will result in decrease in erythrocyte number. There has, however, been no confirmation of their theory concerning the presence of a specific hemopoietin. Mansfield[8] working with thyroidectomized animals, some of which were experimentally rendered anemic, and some transferred to high altitudes, came to the conclusion that the thyroid gland was necessary for the proper functioning of the erythrocytopoietic tissues. Dallwig, Kolls and Loevenhart[9] demonstrated that oxygen lack, or fall in oxygen tension, is an effective stimulus to the red bone-marrow, resulting in increased erythrocyte production. Drinker[10] in a recent review of the subject states that at present the idea, unsatisfactory as it is, of oxygen want as the stimulus for the production of red blood-corpuscles must be accepted.

In any experimental analysis of the factors determining erythrocytopoiesis, a clear distinction must be made between (1) the fundamental stimulus (or stimuli) which, by disturbing the balance of normal metabolism, causes increase or decrease in erythrocytopoietic activity; (2) the basic morphological and physico chemical conditions which are necessary for the successful operation of the fundamental stimulating agent. To the second category belong the spatial conditions of "intravascular location" with slow blood flow, and "favorable relation to transudations from the blood plasma."

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[7] Carnot, P., and Deflandre, C., *Compt. rend. de la Soc. biol.*, 1906, cxliii, 384.

[8] Mansfield, G., *Pflüger's Arch.*, 1913, clii, 23.

[9] Dallwig, H. C., Kolls, A. C., and Loevenhart, A. S., *Am. J. Physiol.*, 1915, xxxix, 77.

[10] Drinker, C. K., *Oxford Med.*, 1920, ii, 509.

From several lines of study, data have been obtained which suggest the nature of the fundamental stimulating agent, given favorable spatial conditions, which operates to effect erythrocytopoiesis. Our data are derived, (1) from a study of the erythrocytopoietic activity accompanying thyroid-accelerated metamorphosis in the larval frog; (2) from a study of the normal seasonal erythrocytopoietic variation (response to temperature changes) in the adult frog; and (3) from a study of the erythrocytopoietic effect following the creation of an erythrocyte deficiency in the frog by means of cardiac aspiration or saponin treatment.

After thyroid treatment of frog larvæ the erythrocytopoietic activity of the spleen is markedly increased.[11] The mechanism by which this result is brought about appears to be as follows: (a) The thyroid extract causes increase in general metabolic rate. (b) This leads to increased formation of acid metabolites including especially carbon dioxide (and other waste materials of cell activity), with resulting increased hydrogen ion concentration. Helff[12] has shown that the carbon dioxide—oxygen exchange increases steadily during the first week or ten days after thyroid treatment, and our histological studies have shown that there is a marked increase in erythrocytopoietic activity during this time. (c) The increased concentration of carbon dioxide acts as a hormone to stimulate more rapid respiratory rate and more rapid erythrocyte differentiation. Both of these rate changes represent merely two aspects of the general physiological adaptation of the animal to the new high metabolic level.

From the standpoint of "usefulness" it is clear that an increased metabolic rate with resulting increased respiratory and erythrocytopoietic rate means: (a) More rapid internal respiration; (b) more efficient transport of oxygen and carbon dioxide between respiratory organ and body tissues; (c) more efficient external respiration. The importance of the erythrocyte, in each of these processes, as furnishing the chief material basis, is obvious. Erythrocytopoiesis is accomplished in those localities which are most favorable for the process, *i. e.*, endothelium-lined spaces where the blood current is slow, as in the sinuses of the spleen, kidney and liver. This differentiation of lymphoid hemoblasts which are in the blood vessels, into erythrocytes is followed by the migration into the blood vessels of other lymphoid hemoblasts, which up to this time have been located extravascularly.

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[11] Jordan, H. E., and Speidel, C. C., *J. Exp. Med.*, 1923, xx, 529.

[12] Helff, O. M., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 34.

Instead of thyroid extract, the extrinsic factor initiating an increase in erythrocytopoiesis may be temperature rise. In the adult frog erythrocytopoietic changes accompany seasonal temperature changes. In winter, during hibernation, the spleen is relatively quiescent, little erythrocytopoiesis taking place. The carbon dioxide output must be coincidentally quite low. With the transition to spring the average level of body temperature again rises, bringing about an increase in metabolic rate. This again initiates the chain of reactions described above; increased carbon dioxide concentration, increased respiratory rate, and increased erythrocytopoiesis. Conversely, in the transition period from late summer to autumn the temperature falls, bringing about a decrease in metabolic rate. This means a decrease in carbon dioxide formation and in rate of respiration, with consequent lessening of the stimulus for erythrocyte differentiation. As a result, many lymphoid hemoblasts and their lymphocyte precursors fail to differentiate into erythrocytes, thus adjusting the rate of erythrocytopoiesis to fall conditions. In many autumn frogs (28 out of 75) the spleen shows a variable number of prominent white nodules. Upon microscopic examination these are found to be areas of degenerating lymphocytes. In others the adjustment may be made without this wholesale disintegration of lymphocytes. Such nodules have not been seen in our spring specimens.

Experimental hemorrhage brought about by aspiration of blood directly from the heart of the frog causes an erythrocyte (and plasma) deficiency which results in a stimulation of the rate of erythrocytopoiesis.[13] Both erythrocytes and blood plasma function in the transportation of carbon dioxide. A deficiency of these leads to greater concentration of carbon dioxide, with resulting stimulation of the erythrocytopoietic tissues, until a more normal carbon dioxide balance is regained. A similar result follows administration of the hemolytic toxin, saponin.

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[13] Jordan, H. E., and Speidel, C. C., *Am. J. Anat.*, 1923, xxxii, 155.

An experiment by Danchakoff[14] bears on our problem. She finds that an intense stimulation of erythrocytopoiesis occurs in the spleen of the chick embryo if a spleen graft is made on the allantois at the ninth day of incubation, a time when the vascular development of the spleen primordium renders it suitable for erythrocytopoietic activity. On the other hand, if the graft is made at an earlier date, before the spleen primordium has become vascularized, there results a defective system of splenic vessels and sinusoids, and a differentiation of the splenic cells into granublasts instead of erythoblasts. In the first case we interpret the erythrocytopoietic stimulation as a result of the increased carbon dioxide concentration in the body fluids of the embryo, following the interference with the respiratory and circulatory functions of the allantois by the graft. In the second case, although the allantoic functions have been interfered with as before, the vascular development of the spleen has not proceeded far enough for this region to offer a favorable locus for erythrocytopoiesis. The conditions are, however, favorable for granublastic differentiation, *i. e.,* the morphological conditions of extravascular location and relatively meagre blood supply, together with the fundamental stimulus of tissue lysis (proteolysis). This matter is discussed in another paper.[15]

In each of the cases cited, there is an extrinsic or relatively remote factor (the active principle of the thyroid extract, change in temperature, depletion of blood, and injury to the allantois) which has caused a variation in the concentration of carbon dioxide in the blood. It is this change in carbon dioxide concentration which is believed to be the more immediate fundamental stimulating cause of change in erythrocytopoietic rate.

In the experiments of Dallwig, Kolls and Loevenhart[9] the decrease in oxygen tension of the respired air is followed by increased carbon dioxide accumulation. The oxygen lack in the respired air is the more remote factor, the carbon dioxide in the body fluids is the more immediate factor, in bringing about the increased erythrocytopoietic activity in the bone marrow. As to just what the mechanism is by which carbon dioxide acts to stimulate the erythrocytopoietic tissues nothing is known. Dallwig, Kolls and Loevenhart, finding also that carbon dioxide concentrations of from 0.5 per cent to 1.0 per cent cause some stimulation of the bone marrow, suggest that the mechanism of this stimulation is to be found in the acid properties of carbon dioxide and its consequent power to decrease oxygen fixation.

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[14] Danchakoff, V., *Am. J. Anat.,* 1916, xx, 255.

[15] Jordan, H. E., and Speidel, C. C., *Am. J. Anat.,* 1924, xxxiii (in press).

That certain of the nitrogenous waste substances formed in metabolic activity, *e. g.,* ammonia, may be concerned in the stimulation of erythrocytopoiesis must be admitted as possible. However, certain other observations have led the writers[15] to the provisional conclusion that the products of protein metabolism constitute the fundamental stimulus that induces a lymphoid hemoblast to differentiate into a granular leucocyte. Thus, the fundamental stimulus for erythrocytopoiesis is regarded as coming from carbohydrate and fat metabolism, the stimulus for granulocytopoiesis as coming from protein metabolism.

Given lymphoid hemoblasts in suitable locations differentiating at a certain rate into erythrocytes, the data from the rather extreme conditions studied suggest that an increased concentration of some product of cellular metabolism, probably carbon dioxide, accelerates the rate of erythrocytopoiesis, decreased concentration retards it. The inference follows that in normal metabolism the more immediate fundamental stimulus for erythrocytopoiesis is the presence of a certain concentration of this metabolite.

202 (2434)

What determines the color of the light of luminous animals?

By E. NEWTON HARVEY.

[*From the Physiological Laboratory, Princeton University, Princeton, N. J.*]

A luminous ostracod (species unknown) occurs in the sea about Jamaica, B. W. I., whose light is more yellow than the Japanese ostracod, *Cypridina hilgendorfii.* Like the Japanese form, the Jamaican form exhibits the luciferin-luciferase reaction, *i. e.,* a hot water non-luminous extract of the luminous gland allowed to cool (containing luciferin), will give light when mixed with a cold water extract of the luminous gland allowed to stand until its luminescence completely disappears (containing luciferase). Moreover, luciferin from the Jamaican species will luminesce if mixed with the luciferase of the Japanese species, and *vice versa.* By carrying out the four possible *"crosses"* one may determine whether the color of the resulting light is controlled by the ostracod supplying the luciferin or the luciferase.

The following results were obtained:

Japanese luciferase x Japanese luciferin—bluish light.
Jamaican luciferase x Japanese luciferin—yellowish light.
Jamaican luciferase x Jamaican luciferin—yellowish light.
Japanese luciferase x Jamaican luciferin—bluish light.

It is apparent that the luciferase determines the color of the light.

203 (**2435**)

The influence of volume of culture medium and cell proximity on the rate of reproduction of Protozoa.

By WILLIAM E. GREENLEAF.

[*From the Osborn Zoological Laboratory, Yale University, New Haven, Conn.*]

Experiments were undertaken to determine (1) the relationship between the volume of culture fluid and the division rate of Infusoria, and (2) the effect of cell proximity on the division rate. The Infusoria studied were pedigree races of *Paramecium aurelia, Paramecium caudatum,* and *Pleurotricha lanceolata.* The experiments comprised four lines of each species bred in 2, 5, 20 and 40 drops of hay infusion. Forty drops were approximately equal to 1 cc. At the end of five day periods the average per diem division rate was obtained for the four lines in each volume.

In the set of experiments with different volumes of culture medium, the average per diem rate was higher in the larger volumes, as shown by the following data, based on the averages for twenty-seven experiments lasting through a period of five days each for the three species mentioned, or nine experiments per species: animals in 2 drops, 0.92 division; in 5 drops, 1.03 divisions; and in 20 drops, 1.20 divisions. Eighteen of the experiments, or six experiments per species, included four lines each in 40 drops with an average per diem division rate of 1.27. Woodruff[1] in his studies on the effect of excretion products of Protozoa on their rate of reproduction obtained equivalent results and interpreted the lower division rate in smaller volumes of medium as due to the effect of the excretion products of the animals.

The second set of experiments differed from the above in that it was confined to a pedigree race of an hypotrichous infusorian and, instead of one animal, two animals were bred together on each slide, in order to determine whether, as Robertson[2] maintained in his studies on *Enchelys farcimen* Ehr., cells mutually accelerate their division rate by the production of a chemical agent or "autocatalyst". At the beginning of each experiment single sister cells were isolated and carried simultaneously as controls. The average per diem division rate for two animals, computed on the one animal basis, in sixteen experiments of this type was below that for one animal in the corresponding number of drops, as follows: 1 animal in 2 drops, 1.35 divisions; 2 animals in 2 drops, 1.03 divisions; 1 animal in 5 drops, 1.81 divisions; 2 animals in 5 drops, 1.54 divisions. The division rate in five drops in each instance was the more rapid, and further, there was a smaller difference between the average per diem division rate of the one animal and two animals in the five drops than in the two drops.

In brief, the data from both sets of experiments are entirely concordant and afford not only no evidence that the cells produce a substance which accelerates the division rate, but also supply further evidence that the excretion products of the cells are inimical to reproduction.

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[1] Woodruff, L. L., *J. Exp. Zoöl.*, 1911, x, 557; *J. Exp. Zoöl.*, 1913, xiv, 575.

[2] Robertson, T. B., *Biochem, J.*, 1921, xv, 595; *J. Physiol.*, 1922, lvi, 404; *The Chemical Basis of Growth and Senescence*, Philadelphia, 1923, 81-105.

204 (2436)

Visualization of the gall bladder of the dog by the Roentgen ray.

By HAROLD A. ABRAMSON.*

[*From the Laboratories of the Department of Surgery, Columbia University, New York City.*]

By injecting solutions of sodium iodide or Neo-silvol (Parke-Davis) into the gall bladder of the dog, the movements of the gall bladder and the fate of contained fluids may be easily studied fluoroscopically and by radiograph. Neo-silvol, a silver-protein-iodide compound, is particularly useful because it is not readily absorbed and is relatively non-irritating. Excellent radiographs of the gall bladder have been obtained after direct injection of 1 cc. of a 35 per cent solution of sodium iodide. The volume of the gall bladder in a medium sized dog is greater than 2½ cc. Much more of the salt may therefore be used, and isotonicity still be preserved since sodium iodide is isotonic at about a concentration of 14½ per cent. Proportionate amounts of Neo-silvol may similarly be used. The presence of pneumoperitoneum remaining postoperatively or induced experimentally, is a distinct aid in visualization where it is desirable to use small concentrations of material.

The following observations have been made on six dogs while recovering from anesthesia, after anesthesia, or a number of days after operation. The total number of actual experimental hours of observation was between fifteen and twenty.

1. *Spontaneous Expulsion.*

Only once during the course of this series was there seen spontaneous expulsion of material from the gall bladder. This was in a cholecystostomized dog (No. 3361, May 29, 1923) which five days after a small bored rubber tube had been placed in the gall bladder wall, was taken to fluoroscopy. Four cc. of 150 per cent sodium iodide was injected through the tube. The gall bladder became beautifully outlined. During the next ten minutes the dog was standing comfortably on the platform on four legs, or occasionally sitting down, nothing was seen to enter the duct and go into the duodenum. After that period the material inside the gall bladder was then seen to distend the cystic and common duct, and several globules of sodium iodide left the common duct and passed into the duodenum, moving slowly, propelled by peristalsis of the gut. The ducts then disappeared from view. For the next half hour nothing more left the gall bladder. Since actually observed material, estimated at 3 cc., left the gall bladder, the gall bladder contracted, actively or passively, expelling the mentioned volume. It must be remembered, however, that this was observed five days after cholecystostomy had been performed, and there was a tube still through the wall of the gall bladder.

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*Introduced by Reuben Ottenberg.

2. *Pressure on the abdominal wall.*

In both intact and cholecystostomized gall bladders very slight pressure on the abdominal wall, laterally or antero-posteriorly, did not force sufficient material into the duodenum to make visualization possible. With increasing pressure, increasing amounts of the contained solution could be forced until very little remained and the collapsed, flaccid gall bladder was distinctly seen.

3. *Movements of the animal.*

Sitting up, lying down, or moving about by the dog, never resulted in the expulsion of the material in gall bladder in sufficient quantity to be seen by the fluoroscope.

4. *Respiration.*

At no time could respiration be said to cause enough material to leave the gall bladder to show fluoroscopically in the duodenum. While it may be said that very minute amounts might have been forced out and not visualized, it is justifiable to state that if any had come through, in all probability it would have accumulated at some point at some time and would have been seen. At every fluoroscopic examination the patency of the ducts was tested with pressure on the abdominal wall. However, this much must be said in favor of the view that the change in intra-abdominal pressure produced by respiration forces material from the gall bladder. During respiration and with each inspiration there was a slight flattening along the longitudinal axis of the gall bladder. Hence with an organ whose internal pressure is greater than that in the duodenum, with a relaxed sphincter of

Odi, it is conceivable that the change in shape might produce an overflow of contained fluid. It is important to think of the physics of the biliary tract as well as its physiology. The gall bladder is a markedly distensible bag, connected to the biliary system by a tube, the cystic duct, to the common duct and the hepatic ducts. At any moment there is a definite "cholestatic" pressure in these ducts and the gall bladder. The pressure in the duodenum may be considered almost proportional to that produced at the sphincter of Odi. Excluding spasms of smooth muscle and obstructive mechanical factors, it is simply obeying fundamental hydrostatic principles to state that when the pressure in the gall bladder is greater than that in the duodenum under the conditions mentioned above, bile will flow from the gall bladder into the duodenum, regardless of contractile power.

The method of study which has been just outlined was developed during an attempt to study the Meltzer-Lyons test fluoroscopically so that the effect of substances on the Ampulla of Vater could be seen directly by observations of material in the gall bladder. These studies are not yet ready to be reported. Quantitative expulsion experiments of Neo-silvol from the gall bladder over a given period of time are also incomplete, as well as the contractility of the gall bladder under increasing intra-gall bladder pressure.

ABSTRACTS OF COMMUNICATIONS.

Seventh meeting.

Peking Branch.
Peking, China, March 10, 1924.

205 (2437)

The value of egg yolk in supplementing diets deficient in calcium.

By ERNEST TSO (by invitation).

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

According to McCollum[1] the deficiency of calcium in eggs is a serious limitation of their value as a supplementary or protective food.

On a diet containing 398 mg. of calcium per 100 gm. of ration[2] which was considered to be markedly deficient in calcium, young rats failed to grow normally in spite of the presence of 5 per cent of butter fat. On the other hand, the addition of 1 to 2 per cent of cod liver oil to the same diet was found to be strikingly more effective in stimulating growth and "protecting young animals against the harmful effects of partial calcium starvation."

More recently Bethke, Steenbock, and Nelson[3] have correlated the calcium requirement of growing rats to the intake of cod liver oil or the vitamins contained therein. These investigators found "that no amount of Ca added to the basal ration without vitamin addition was effective in increasing growth, but when cod liver oil was added progressively in increasing amounts, less and less Ca was required for normal growth."

It is highly significant to compare these observations with the striking results I have obtained from feeding albino rats simply on egg yolk and millet. The main diet consisted of 30 parts by weight of fresh egg yolk and 70 parts ground millet seed *(Setaria*

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[1] McCollum, E. V., *Newer Knowledge of Nutrition*, 1922, pp. 171 and 343.

[2] Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, N., *Am. J. Hyg.*, 1921, i, 524.

[3] Bethke, R. M., Steenbock, H., and Nelson, M. R., *J. Biol. Chem*, 1923, lviii, 71.

italica). 100 gm. of this food mixture contained approximately 70 mg. of calcium.

A control diet, comparable with the egg yolk and millet ration in proportions of fat, carbohydrate, and protein, was made up of 20 parts by weight of whole milk powder (Merrell-Soule), 5 parts of butter, and 75 parts of millet seed. 100 gm. of this food contained 220 mg. of Ca.

As fed to the animals 100 gm. of each ration were cooked in 110 cc. of distilled water for 15 minutes or until the mixture had thickened into a pudding consistency and the raw odor of the cereal had disappeared. The food as well as additional amounts of distilled water was fed *ad lib.* and daily replaced.

On the egg yolk and millet diet male rats grew to the average weight of 260 gm. and female rats, 170 gm. in 20 weeks. One female rat had a litter of 8 young; however, none survived beyond the first week. The male animals on the milk diet grew somewhat more vigorously; their average weight was 280 gm. One female rat gave birth to 7 young. One of these died on the third day. The rest weighed on the average 22.6 gm. each at the age of eighteen days.

The results of these preliminary experiments seem to indicate that egg yolk given in equivalent amounts in terms of caloric values, is nearly as efficient as milk in promoting normal growth in young rats, notwithstanding the wide difference in calcium content between the two rations. Like cod liver oil, egg yolk is evidently possessed of a special property of increasing the Ca assimilation and thereby sparing the excess of Ca intake which is otherwise required in the food.

However, the Ca intake in the egg yolk and millet diet is obviously close to the minimum level and inadequate to meet the needs of lactating animals.

206 (2438)

The localization of Bacillus proteus x 19 in the subcutaneous tissues of the guinea pig by means of preliminary injections of carbon at different intervals.

By RALPH G. MILLS, CLIFFORD L. BARTLETT, and AN-CH'ANG LIU.

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

1. The subcutaneous injection of *Bacillus proteus* x 19 into guinea pigs caused death from acute septicemia in a considerable percentage of cases; when a suspension of carbon was simultaneously introduced the mortality was definitely reduced, but when the injection of carbon preceded that of the bacteria by an appreciable interval this initial mortality was reduced to zero in a series of 61 animals. In an additional group of 9 animals in which the carbon and proteus were injected together there were no deaths, but among 21 guinea pigs in which the organisms were introduced without carbon, there were three deaths from septicemia.

2. The only obvious explanation for the absolute protection afforded these animals is a mechanical one, *viz.*, a blockade of the lymphatic drainage of the injection site.

3. The protection given by the lymphatic blockade is evidently a relative one, and depends more or less upon the limitation of the organisms to the injection area. Invasion of the blood stream, even in sub-lethal doses, was observed only in 7 instances, 4 in which the injection of carbon preceded that of the proteus by an interval of less than five hours, 1 in which the two suspensions were given simultaneously and 2 in which the organisms were injected alone. Complete protection was apparently afforded when the interval was sufficiently long, *i. e.*, five hours or more, allowing complete obstruction of the lymphatics to take place. The bacterial suspension evidently diffused somewhat beyond the actual point of injection, but was really limited by the obstruction of lymphatics over an area somewhat larger than that in which edema could be observed. Lines of carbon were seen stretching as far as 2 to 3 cm. from the point of actual injection.

4. The conclusions reached in the previous study and the observations recorded were confirmed and in some respects elaborated by these additional experiments. The virulence of the strain used was shown to be preserved during the interval between the two studies, and cultivation in a carbon lesion in the guinea pig and by passage through the eye of a rabbit failed to increase this virulence materially.

5. The agglutinative power of the serum from all animals was tested at different intervals and no constancy in its appearance, intensity or persistence was demonstrated. In a given individual it was practically independent of the intensity of the local or general reaction following injection and of the state of nutrition represented by interruption of expected weight increase, but somewhat increased by the persistence of bacteria in the lesion. The presence of carbon appeared to create conditions favorable for the production of agglutinins.

6. A sharp rise in temperature followed the injection of the organisms within two hours in all animals, representing the peak of the curve in a large majority. In almost every case there was a return to normal by the third day. This rise of 1 to 2° above that incident to the excitement created by the injection procedure was definitely referable to the effect of the introduction of the bacteria, but its exact cause is unknown.

7. Loss in weight following injection was observed in most animals, in some instances it was extreme. The loss was greatest and was made up more slowly when the injection of the bacteria followed that of carbon by a considerable interval. This was presumed to represent the influence of the bacteria persisting under the protection of the carbon.

8. Organisms were demonstrated in the lesions in 28 out of 38 surviving animals at least forty days after injection. The persistence was apparently not related to the manner in which the original injections had been made.

207 (2439)

The influence of experimental ascites on the diuretic action of drugs.

By CARL F. SCHMIDT and K. K. CHEN.

[From the Laboratory of Pharmacology, Peking Union Medical College, Peking, China.]

In a brief series of experiments[1] it was found that experimental ascites in dogs diminished or suppressed urine flow, apparently because of a simultaneous rise in pressure within the renal vein. The influence of similar procedures on the action of diuretic drugs has been investigated in 15 anesthetized dogs, with the uniform result that small doses of saline or xanthine diuretics were unable to cause diuresis until abdominal pressure was lowered. Larger doses of salines were usually effective against a suppressing abdominal pressure, while the xanthines were ineffective in any dose. Sodium sulfate was more effective than sodium chloride or bicarbonate.

The method seemed to be applicable to estimating the relative efficiency of diuretic drugs in the presence of a controllable degree of venous engorgement in the kidney, provided that the effects of experimental ascites on urine flow are due only to high pressure in the renal vein. By a perfusion experiment, using citrated blood, in which the kidney, its vessels, and the ureters were subjected to external hydrostatic pressure, it was found that an external pressure of 27.7 mm. of mercury caused suppression of urine when the renal vein was exposed to the pressure, but when the renal vein was protected from collapse by a glass catheter an external pressure of even 69 mm. had only slight effect on urine flow. It was clear that the phenomenon under observation was due largely, if not wholly, to the rise in venous pressure.

To estimate the relative efficiency of the drugs, the salines were injected in M/7 solution with an abdominal pressure just sufficient to suppress urine, the criteria being the extent of diuresis and the additional abdominal pressure required to suppress urine after the drug. Sodium phosphate, sulfate, nitrate, bicarbonate, chloride, and acetate, as well as urea and glucose, were compared, and caffeine, theobromine, and theophylline were also tried. Pituitary extract and digitalis failed to cause diuresis.

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[1] Thorington, J. M., and Schmidt, C. F., *Am. J. Med. Sci.*, 1923, clxv, 880.

The results indicated that phosphate, sulfate and urea were by far the most effective; nitrate was intermediate; glucose and bicarbonate were weaker, but stronger than chloride; acetate was the weakest saline.

Any saline could bring out urine against a suppressing abdominal pressure, while equal or larger volumes of distilled water could not. The xanthines were all ineffective in any dose against high abdominal pressure, though definitely diuretic with lower pressure. Large doses of theocine usually caused permanent suppression of urine, caffeine was less harmful, and theobromine usually harmless, though none was effective until abdominal pressure was reduced.

These differences in degree of effectiveness seem to depend on the fact that the most effective drugs—phosphate, sulfate, urea—are non-threshold salines, and would therefore raise pressure in the tubules more than the other salines which are more readily reabsorbed. Any saline decreases the osmotic resistance to filtration or secretion, as pointed out by Cushny[2] and can therefore raise tubular pressure if enough is given to compensate for rapidity of reabsorption. Richards and Schmidt[3] found that any diuretic brought into play more functional units in the frog's kidney. It seems probable that this is the only effect of the xanthines; salines have a similar effect and in addition decrease osmotic resistance to secretion; the non-threshold salines also decrease reabsorption from the tubules and are therefore most effective in the presence of venous engorgement.

The lack of diuresis and the possibility of suppression of urine by large doses of xanthines when abdominal pressure is high indicates that these drugs should not be given in ascites until pressure has been reduced by paracentesis.

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[2] Cushny, A. R., *The Secretion of the Urine*, London, 1917, p. 121.

[3] Richards, A. N., and Schmidt, C. F., *Am. J. Physiol.*, 1922, lix, 489.

ABSTRACTS OF COMMUNICATIONS.

Eleventh meeting.

Western New York Branch.
Geneva, New York, April 5, 1924.

208 (2440)

Effects of dosage and previous diet on blood sugar curves in sheep after intravenous administration of insulin.

By AARON BODANSKY (by invitation).

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

Sheep do not show any convulsive symptoms or visible distress after intravenous administration of insulin even when the blood sugar is depressed to 30 mg. per 100 cc. It is possible that the convulsive level for sheep would be lower, but so far we have been unable to reach it. With increased dosage the effect is not so much upon the intensity as upon the duration of the hypoglycemia.

It has been shown[1] that recovery from the lowest sugar value begins immediately after it has been reached, if about 5 units per 100 pounds are injected intravenously.

When 10 units were administered, the hypoglycemia, at or near the lowest level, lasted about one hour. When 15 units were injected, the low level was maintained about two to three hours.

Thyroidectomized sheep have been reported[2] to show a similar flat bottom in their insulin blood sugar curves after the small dose (5 units) which failed to produce it in the normal animal. In the light of the evidence then available, it was suggested that this effect of thyroidectomy might be due to the lack of the thyroid secretion, assumed to aid normally in the control of the blood sugar through its effect upon glycogenolysis.

The standard conditions of the experiments included an interval of about eighteen hours between the last meal of the sheep and the beginning of each experiment.

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[1] Bodansky, A., PROC. SOC. EXP. BIOL AND MED., 1923, xx, 538.

[2] Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 46.

This interval was varied and a longer fast was instituted. Under the changed conditions, the normal sheep showed a flat bottom in the insulin blood sugar curve after the administration of the standard dose.

On the other hand, when the thyroidectomized sheep were allowed free access to food during the night preceding the experiment, blood sugar curves were obtained indistinguishable from the curves of normal animals which had been given the same dose of insulin but had been submitted to the standard eighteen hours' fast.

Further observations are in progress—of the effect of dosage and of diet, and of the relative importance of the general cachectic factor and of the specific endocrine deficiency in the control of the blood sugar after insulin administration.

The insulin for these experiments has been obtained through the courtesy of Eli Lilly and Company.

The work was carried out under a grant from the Heckscher Research Foundation.

209 (2441)

The influence of insulin on the liver glycogen of animals poisoned with phlorhizin.

By CARL F. CORI.

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

It has been shown previously[1] that insulin produces a marked diminution in the sugar excretion of rabbits completely poisoned with phlorhizin, whereas the nitrogen excretion during an observation time of four hours is not appreciably diminished. It was concluded that insulin does not directly influence the sugar formation from proteins during phlorhizin poisoning. Since a rather large amount of sugar is retained when phlorhizinized rabbits are treated with insulin, it was assumed that part of this sugar is stored as glycogen. This seemed the more probable, since Ringer,[2] in a recent publication, showed that completely phlorhizinized dogs which excrete less sugar during the action of insulin give rise to the excretion of extra sugar when the action of insulin has worn off.

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[1] Cori, C. F., *J. Pharmacol. and Exp. Therap.*, 1924, xxiii, 99.

Six experiments were undertaken in which four rabbits and two cats were used. In all animals glycogen synthesis could be demonstrated. The glycogen was determined by Pflüger's method or by the total carbohydrate method. One protocol is given below. The experimental methods have been described elsewhere.[3]

Rabbit 3. February 1, 1924, abdominal window established; food withdrawn. February 4, first phlorhizin day. February 5, second phlorhizin day. February 6, day of experiment.

Time	Blood sugar mg. per 100	Total carbohydrates in liver gm. per 100	Sugar : Nitrogen ratio in urine	Amount of liver taken in gm.
9:30	105	0.87	2.78	1.99
9:40	4 units insulin (Lilly) subcutaneously			
12:40	92	1.80	2.53	2.07
1:00	4 units insulin subcutaneously			
3:40	88	3.61	1.91	2.16
8:00	76	3.25	2.58	1.46
The next day				
12 a. m.	118	1.39	3.35	3.14

As will be seen from this table a marked glycogen synthesis occurs, which reaches its maximum in six hours, while the D:N ratio falls to 1.91. After the insulin injections have been discontinued the glycogen accumulated in the liver disappears gradually again, giving rise to extra sugar in the urine.

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[2] Ringer, M., *J. Biol. Chem.*, 1923, lviii, 483.

[3] Cori, C. F., Cori, G. T., and Pucher, G. W., *J. Pharmacol. and Exp. Therap*, 1923, xxi, 377.

210 (2442)

The influence of insulin on the liver glycogen and free liver sugar of starving animals.

By CARL F. CORI.

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

Dudley and Marrian[1] reported diminution in the liver glycogen in one rabbit and in several mice during the convulsive stage of insulin action. Since it was thought that convulsions may produce changes which are not necessarily related to the action of insulin, experiments were performed with animals above the convulsive blood sugar level.

Eight rabbits and fifty mice were used. It was found in rabbits that there is no appreciable change in the glycogen (Pflüger's method) or total carbohydrate content of the liver within the first hour of insulin action, whether the initial glycogen content of the animal was high or low, or whether the fall in blood sugar was slight or great. In the second to sixth hour of insulin action, the glycogen content of the liver may remain constant or may decrease. Thus in three experiments the liver glycogen did not change during the periods of observation of three, four, and six hours respectively, while in three other experiments the glycogen was lowered during the second hour of insulin action. The initial glycogen content in the latter group of experiments was higher than in the former. In the experiments with mice, the fact that they live only two days when deprived of food, was taken into consideration. Consequently starvation times from one to three hours were chosen. The length of time that insulin was allowed to act varied from fifteen minutes to one hour. The livers were analyzed for glycogen by Pflüger's method and the blood sugar of each mouse was determined. An equal number of controls and injected mice was used. The following averages were obtained: 1.47 per cent liver glycogen, 0.131 per cent blood sugar in control mice, 0.91 per cent liver glycogen, 0.066 per cent blood sugar in insulin mice. In only one-half of the experiments performed was the glycogen content of the liver of the injected mice lower than that of the corresponding controls. This group of mice had a higher initial glycogen content than the group of mice in which no change in glycogen content was observed after insulin. It was found that the blood sugar level at which convulsions in mice occur is around 0.045 per cent.

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[1] Dudley, H. W., and Marrian, G. F., *Biochem. J.*, 1923, xvii, 435.

In the experiments on the influence of insulin on the free liver sugar, two rabbits and thirty mice were used. It was found that insulin lowers the free liver sugar of both starving rabbits and mice. Thus the free liver sugar in fifteen control mice was as an average 0.52 per cent, while in fifteen mice injected with insulin, in which the blood sugar ranged from 0.094 to 0.045 per cent the free liver sugar was 0.31 per cent.

It is assumed that in those instances in which the liver glycogen diminishes under the influence of insulin, a special form of glycogenolysis occurs which does not have sugar as its end-product. In those types of glycogenolysis which give sugar as an end-product, as epinephrine glycogenolysis for example, the free liver sugar[2] and the output of sugar by the liver into the blood stream was found to be increased, while it has been shown that insulin diminished both the free liver sugar and the output of sugar by the liver into the blood stream.[3] It is believed that this special form of glycogenolysis is in some way connected with the glycogen synthesis, that is produced by insulin, whenever there is an excess of sugar in the body, whether this sugar is derived from administration from without or from excess production in the body itself (compare preceding abstract).

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[2] Cori, C. F., Cori, G. T., and Pucher, G. W., *J. Pharmacol. and Exp. Therap.*, 1923, xxi, 377.

[3] Cori, G. F., Cori, G. T., and Goltz, H., *J. Pharmacol. and Exp. Therap.*, 1923, xxii, 355.

211 (2443)

A paradoxical stability phenomenon with spontaneously agglutinating diphtheria bacilli and its practical import.

By R. R. MELLON, W. S. HASTINGS, and C. ANASTASIA.

[*From the Department of Laboratories, Highland Hospital, Rochester, N. Y.*]

One of the interesting and perhaps practical outgrowths of our inquiry into the rôle of surface tension in the stability of bacterial suspensions[1] is indicated by the title. Briefly, it has been found possible to stabilize certain strains, *e. g.*, in 0.85 per cent NaCl, in which immediate and complete flocculation results, if the conventional methods of preparing the suspensions are employed.

The strain is readily emulsified in 2.5 per cent NaCl and when this is then diluted with two volumes of distilled water making the final concentration about 0.8 per cent, or with four volumes making it 0.5 per cent, the organism is then quite stable for serological work. With $MgCl_2$ and Na citrate the same procedure is adequate. A few other strains of this group appear to behave similarly. How general the application of the procedure will be cannot now be stated.

The procedure was tried experimentally on the basis of consideration involved in Gibb's Law which indicates an inverse reciprocal relation between interfacial surface tension and adsorption. Thus the surface concentration of solute is much greater than in the bulk of the liquid when the interfacial tension is low, and an additional excess is deposited by the forces of electrical adsorption. It seemed possible then to dilute the solute present in the bulk of the solution more rapidly than that at the surface. Experiment justified the theory, although it may turn out that some other factor not now considered is also active.

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[1] Mellon, R. R., Hastings, W. S., and Anastasia, C., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 279.

212 (**2444**)

A photographic method for studying the growth and nutrition of children.

By H. D. CLOUGH.

[*From the Department of Vital Economics, University of Rochester, and Hillside Home, Rochester, N. Y.*]

The method consists in making stereoscopic photographs of growing children, under standardized conditions, at three month intervals. The apparatus consists of a portable framework for holding the camera, backgrounds, lights, measuring scales, etc., so that all of the physical conditions are kept constant. A plate glass mirror, arranged at the side of the subject at an angle of 45°, serves to give a lateral view, thus making possible the securing of simultaneous anterior and lateral views.

Standardization of position is secured by placing the subject with the following four points resting against a vertical steel rod bearing a centimeter scale: occiput, spine between scapulæ, sacrum, and heels. An adjustable square, sliding on the vertical steel rod, indicates the subject's height on the centimeter scales.

Artificial light (2000 Watts) of such intensity is used as to permit instantaneous photographs to be secured with 1/5 second exposure at F-8. The development of negatives is standardized by using the tank factorial system. All data pertaining to the subject, such as number, date of birth, date of photograph, and weight, are photographed on the film. The photographs are secured with great rapidity.

The method permits visualization of growth and nutritional status and makes it possible for accurate measurements to be made on the anterior and lateral silhouettes for the purpose of developing correlations between the growth of certain parts and other factors such as height and weight.

Stereoscopic prints and stereoscopic projection by means of a double lantern with complementary red and blue-green filters make possible complete visualization of form at each growth stage. This, together with prints from superimposed negatives, makes possible the formation of many concepts which cannot be expressed by anthropometric measurements.

213 (2445)

An attempt to correlate the growth of institutional children with the food supply.

By KATHERINE L. FOSTER and JOHN R. MURLIN.

[*From the Department of Vital Economics, University of Rochester, and Hillside Home, Rochester, N. Y.*]

Hillside Home is an institution consisting of four cottages for boys, three for girls and one for children of both sexes under five. Each cottage except the last contains on the average from 20 to 25 children of mixed ages ranging from six to sixteen. Each has its own kitchen, a cook and house mother. Food is obtained by requisition from a central storeroom and from the Home farm which maintains a herd of cows, a truck garden, small fruit, etc.

From June, 1921, to June, 1923, eight surveys, covering periods of from two to six weeks, were made at nearly uniform intervals of the food consumption in each cottage. Calculations of the energy consumption per person per day were then compared with the total energy requirement for populations of like age distribution, as postulated by Holt and Fales.[1] The average food consumption varied from this requirement in different cottages from a deficit of 403 calories to a surplus of 48 calories per person per day. The average cottage showed a deficit of approximately 200 calories. The average growth in weight varied in different cottages from 81 to 120 per cent of the normal rate of gain as determined by Baldwin[2]—the average of all being very close to the rate of gain found by this author. The conclusion may be drawn that normal growth, as nearly as it can be denominated at present, is possible on an average total energy supply which may vary considerably from the Holt standard.

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[1] Holt, L. E., and Fales, H. L., *Am. J. Dis. of Child.*, 1921, xxi, 1.

[2] Baldwin, B. T., *J. Am. Med. Assn.*, 1924, lxxxii, 1.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

One hundred and fortieth meeting.

Yale University, New Haven, Conn., May 24, 1924.
President Jackson in the chair.

214 (2446)

Changes in the composition of the urine after muscular exercise.

By D. WRIGHT WILSON, W. L. LONG, H. C. THOMPSON and SYLVA THURLOW.

[*From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pa.*]

The effect of short periods of strenuous exercise on the composition of the urine was studied. The urine from normal men was collected in ten minute periods and after a suitable fore-period stair running for one or two minutes was introduced and the collections of urine continued. It was hoped in this way to learn the rapidity and the extent of the response of the kidney to the chemical and physical changes taking place in the organism. There was a marked fall in the urine volume after exercise which could not be overcome by drinking small quantities of water at frequent intervals. A considerable increase in the excretion of acids and conservation of base were demonstrated by the rapid increase in the hydrogen ion concentration, titratable acidity, ammonia and ratio of ammonia to total nitrogen of the urine. The inorganic phosphates increased parallel with the acid excretion. The values reached a maximum in twenty minutes and returned to normal in less than an hour. The hydrogen ion concentration frequently returned to normal more slowly and the phosphate excretion always fell below normal toward the end of the experiment. There was a marked fall in chlorides which apparently was not due to the decreased fluid output or to the loss of chlorides in the perspiration. The excretion of chlorides fell to a minimum in twenty minutes after exercise and then returned to normal. There was a considerable fall in the total nitrogen excretion but no characteristic change in creatinine due to the exercise. The changes observed in the composition of the urine, when compared with the variations in the composition of the blood observed by Barr and his collaborators, indicate that the kidney responds rapidly and efficiently to aid in the neutrality regulation in the body.

215 (2447)

The excretion of lactic acid in the urine after muscular exercise.

By S. H. LILJESTRAND and D. WRIGHT WILSON.

[*From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pa.*]

To study the excretion of lactic acid after exercise, urine was collected in ten minute periods and was analyzed for lactic acid by Clausen's method. After one or two minutes of strenuous exercise the lactic acid excretion rose from a normal of about 2 mg. in ten minutes to about 150 mg. in ten minutes. The excretion reached a maximum within twenty minutes after exercise and then fell rapidly. There was a return to normal in about one hour. The experiments were carried out using men as subjects.

216 (2448)

The gaseous exchange in man during short exposures to a low barometric pressure.

By EDWARD C. SCHNEIDER.

[*From the Physiological Laboratory, School of Aviation Medicine, Mitchel Field, N. Y.*]

In four series of experiments conducted under a reduced barometric pressure in a low pressure chamber consistent results have shown that the gaseous metabolism may be for a time reduced by a decrease in available oxygen. In many cases the decrease was not evident until a correction was made for the additional use of oxygen that resulted from the further muscular effort of increased respiration.

The reduction in oxidation was present in one case at a barometric pressure of 530 mm., partial pressure of oxygen 110 mm.; in others at 443 mm., partial pressure 92 mm.; while in one case it began at 317 mm., partial pressure 66 mm.

Observations on the gaseous content of the blood showed that less oxygen was withdrawn from 100 cc. of blood as it flowed through the arm when the individual was under a barometric pressure of 350 mm. than when under 760 mm. The rate of blood flow through the arm was not increased under the low barometric pressure and, in some instances, was somewhat below normal.

With a prolonged exposure to a pressure of 400 mm., the oxidations of the body were at first reduced; but, after a period of several hours, tended to return to or even rise above normal. The increased rate of metabolism appeared to be associated with the onset of "mountain sickness" symptoms.

217 (2449)

Oxidation-reduction equilibria of indophenols.

By W. MANSFIELD CLARK, H. D. GIBBS and BARNETT COHEN.

[*From the Hygienic Laboratory, United States Public Health Service, Washington, D. C.*]

A summary is given of work being reported in detail in *U. S. Public Health Reports.* The equilibrium potentials at 30° of the indophenol system of oxidation-reduction indicators are precisely formulated by the equation:

$$E_h = E_o - 0.03006 \log \frac{[S_r]}{[S_o]} + 0.03006 \log [K_r K_2 (H^+) + K_r (H^+)^2 + (H^+)^3] - 0.03006 \log [K_o + (H^+)]$$

The potentials are determined not only by the proportions of oxidant and reductant but also by the hydrion concentration. The indophenol system lies at a more positive (more oxidizing) range than any other system of indicators studied by us up to this time. Substitutions in the indophenol molecule of halogen or alkyl groups produce rather small changes in the oxidation-reduction potential and relatively more important changes in the acid-base properties of the compounds. The latter permits selection of compounds compatible with physiological ranges of pH, and possibly useful for indicating certain intensities of oxidation-reduction in biological processes.

One such compound is phenol indo-2,6-dibromo phenol, whose acid dissociation constant ($pK_o = 5.7$) is at a point where the dye retains its deep blue color in mildly acid solutions. Many biological fluids reduce this compound in the presence of air.

218 (2450)

Autoplastic and homoplastic parathyroid transplantation.

By **W. W. SWINGLE** and **J. S. NICHOLAS.**

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn., and the School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*]

Six cats were thyroparathyroidectomized and autoplastically grafted with the two external parathyroids. In four animals the glands were implanted into a pocket cut in the sternohyoid muscle; the remaining two cats were transplanted in the *rectus abdominis* muscle. The parathyroids were dissected free from all investing membranes, the dissection being done in a half and half mixture of warm, sterile Ringer's solution and blood serum of the animal from which the glands were taken. In some instances the parathyroids remained in the solution for fifteen minutes before implantation. Table I summarizes the principal results of the experiment.

In a series of homoplastic transplantations, twenty-eight cats were used, the donor of the glands being of the same sex as the recipient, and as nearly the same age as possible. The grafts were of two types with respect to the time when made: (1) immediately following the onset of tetany symptoms; (2) simultaneous or criss-cross operations in which the parathyroids of two cats were exchanged. Tables II, III, IV summarize the chief results of the various types of operation.

TABLE I.

Cat	Location of graft	Symptoms following thyroparathyroidectomy	Recovery	Reextirpation of graft	Result	Remarks
1	Rectus abdominis muscle	Tetany	Complete	36 days after implantation	Fatal tetany	Graft in good condition. Central area replaced by connective tissue.
2	Sternohyoid muscle	Tetany	Complete	33 days after implantation	Fatal tetany	Graft in good condition. Both glands healed in. Central area replaced by connective tissue.
3	Sternohyoid muscle	None	Complete	31 days after implantation	No symptoms	Graft in good condition. Central area replaced by connective tissue. Accessory glands found at autopsy.
4	Sternohyoid muscle	Tetany	Complete	37 days after implantation	No symptoms	Graft in good condition. Central area replaced by connective tissue. Accessory parathyroids probably present.
5	Rectus abdominis muscle	Tetany	Complete	34 days after implantation	Chronic tetany	Graft tissue well preserved. Central area as in other cases. Small accessory parathyroids found in thymus.
6	Sternohyoid muscle	None	Complete	30 days after implantation Glands not found	No symptoms	Graft not located.

TABLE II.

Grafts of the parathyroid into the ear following the onset of tetany.

Cat	Sex	Weight	Symptoms after thyroparathyroidectomy	Grafted	Symptoms	Result	Duration of experiment after grafting	Weight	Remarks
		gm.					days	gm.	
34	♀	550	Tetany and depression	5th day after operation	Tetany and depression	Death	4	450	Graft degenerate. Little vascular connection.
35	♀	820	Tetany and depression	3rd day after operation	Tetany and depression	Death	4	560	Degeneration of graft. Poor vascularization.
37	♀	2250	Tetany	4th day after operation	Tetany	Death	6	1630	Graft degenerate.
38	♀	600	Tetany and depression	4th day after grafting	Tetany and depression	Death	5	502	Graft degenerate.
41	♂	2600	Tetany	7th day after operation	Tetany	Death	6	1000	Graft degenerate.
42	♂	1801	Tetany	2 days after operation	Tetany	Death	4	1740	Graft degenerate.

TABLE III.
Grafts into the Rectus abdominis muscle after onset of tetany.

Cat	Sex	Weight	Symptoms after thyroparathyroidectomy.	Grafted	Symptoms	Result	Duration of experiment after grafting	Weight	Remarks
50	♂	gm. 320	Tetany	24 hours after operation	Tetany and depression	Death	days 3	gm. 290	Degeneration of the graft.
52	♀	420	Tetany	24 hours after operation	Tetany	Death	2	408	Graft not sectioned.
64	♀	2935	Tetany	2 days after operation	Tetany	Death	10	2250	Degeneration of graft.
65	♂	2010	Tetany	24 hours after operation	Tetany	Death	4	1800	Degeneration of graft.
66	♂	2001	Tetany	2 days after operation	Slight tetany	Recovery	32*	1979	Part of graft persisted in fair condition.
68	♂	3660	Tetany	2 days after operation	Tetany and depression	Recovery	36*	3400	Degeneration of graft. Accessory parathyroids probably present.

*Animal succumbed to adrenal insufficiency due to removal of both adrenals. This accounts for the weight loss.

TABLE IV.

Transplantation of parathyroids into the Rectus abdominis muscle before the onset of tetany.

Cat	Sex	Weight	Symptoms	Result	Duration of experiment	Weight	Remarks
		gm.			days	gm.	
53	♂	650	Depression	Death	3	600	Graft poorly vascularized. Not sectioned.
54	♀	1510	Tetany	Death	7	1205	Degeneration of the grafted glands.
55	♀	2420	Slight tetany	Recovery*	Graft removed after 21 days. No symptoms	2020	Parts of grafted glands persisted in fair condition. Accessory parathyroids probably present.
56	♂	5050	No symptoms	Recovery	22	4786	Degeneration of graft.
57	♂	3240	Tetany	Recovery	20	3915	Degeneration of graft. Small accessory parathyroid found in thoracic thymus.
58	♂	3011	No symptoms	Recovery	Graft removed after 10 days. No symptoms	3024	Degeneration of graft. Peripheral border in fair condition.
59	♂	3565	Tetany	Death	7	3430	Degeneration of graft.
60	♂	1210	No symptoms	Recovery	12	1314	Degeneration of graft. Accessory parathyroid found in thymus.
61	♂	2540	Tetany	Recovery	30	2601	Degeneration of graft. Accessory parathyroid tissue not found.
69	♀	3160	No symptoms	Recovery	30	2540	Parts of both grafted glands persisted in fair condition.
70	♂	3120	Tetany	Death	3	3020	Graft not sectioned.
71	♂	2750	Tetany	Death	4	2610	Degeneration of entire graft. Gland area well vascularized.
72	♀	3420	Tetany	Death	4	3360	Grafts made subcutaneously on account of pregnancy. Could not locate the grafts later.
73	♀	1824	Tetany	Death	4	1773	Great degeneration of the grafted tissue. Glands well vascularized.
74	♀	2700	Tetany	Death	7	2100	Degeneration of central area of graft. Peripheral area persisted in fair condition.
75	♀	2250	No symptoms	Recovery	45	2478	Parts of both glands in fair condition.

* It is probable that most of the cats which recovered had accessory parathyroids.

219 (2451)

Observations on the cellular activity in a culture of amphibian liver tissue.

By GEORGE A. BAITSELL.

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*]

On January 2, 1924, two, very large, ameboid cells appeared in a culture of liver tissue, obtained from an adult *Rana pipiens*. This culture had been made on November 25, 1923, some five and one-half weeks earlier, and no cells had previously migrated into the clot. The culture was one of a series of twenty-five, and in none of the others was any cellular activity ever observed. The unusual size and activity of these cells, as well as their migration into the clot after the long inactive period of the tissue immediately commanded attention, and careful observations were made during the succeeding five days in which they retained their activity.

Each of the cell bodies was very irregular and constantly changing in shape, due to a flowing movement of the cytoplasm strikingly similar to that in an ameba. As a result of the cytoplasmic flow, cell processes of various lengths were continually formed at various regions of the periphery. When first observed each of the cells measured about 0.33 mm. in length. Later a maximum length of over 0.5 mm. was attained by one of the cells. The cytoplasm contained many granules of various sizes which moved into or out of the processes with the cytoplasmic current. After five days of continual activity in the clot, both cells rounded off, and each formed a somewhat spherical cell body which measured about 0.15 mm. in diameter. No further movement occurred in either of these cells although the culture was opened and one of them was isolated and placed in fresh medium in a subculture.

The identity of these cells is not clear, and so far as I am aware similar metazoan cells have not been described either in tissue cultures or elsewhere. During the past few years I have observed over a thousand cultures of various adult amphibian tissues, in none of which have cells of this type been present.

Also a great many permanent histological preparations of various amphibian tissues have been studied with the same negative result. The possibility that they might be embryonic in type suggested itself and accordingly a large number of cultures of various tissues from the tadpole have been made and studied. In several of these, cells comparable in size and activity have been found. The experiments are still in progress.

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220 (2452)

The blood platelets in rats on adequate and inadequate diets.

By DAVID N. SHULMAN and LAFAYETTE B. MENDEL.

[*From the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

In view of the fact that Bedson and Zilva[1] disagree with Cramer, Drew and Mottram[2] on the behavior of the blood-platelets in rats deficient in vitamin-A, it was found desirable, in the course of the study of the pathology of avitaminosis, to investigate this disputed question. Applying a method for platelet counting which has for its basic principles the minimum of manipulation and the absence of contact of the drawn blood with possible coagulant surfaces, we have found the blood-platelet content in normal albino rats on adequate diets to approximate 400,000 to 600,000 per cu. mm. We have not observed any significant variation from this average in rats subsisting on diets lacking vitamin-A and showing the typical symptoms of such a deficiency. These observations are essentially different from those of all the above mentioned workers in regard to the normal platelet content of white rats, and confirm Bedson and Zilva's contention that the platelet content is not affected by diets deficient in vitamin-A. The method employed, suggested by Dr. A. B. Dayton's practice on man, is as follows: after the freely protruding tail of the encaged rat is washed, if necessary, with water and dried, a portion of its surface is painted with the diluent and allowed to dry. One of the superficial veins in that area is then punctured with a sharp lancet on which the diluent has also been dried, and as the blood wells up freely it is drawn up into an erythrocyte pipette, the stem of which had previously been filled with the diluent to the 0.5 mark, until the diluent column reaches the 1.0 mark. The diluent is then added to the 101 mark, giving the usual 1:200 dilution. After shaking the pipette for not less than three minutes, a direct count is made of the red blood corpuscles and platelets in the same preparation. The platelets are allowed at least ten minutes to settle. Although the vast majority of the latter are distributed singly, preparations are considered good if not more than six are found in the occasional clump. The primary essentials for consistent count are: a scrupulously clean counting chamber and pipette, the filtering of the diluent[3] immediately before use, the presence of the anticoagulant in the pipette before the entrance of the blood, and a free flow of blood from the wound.

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[1] Bedson, S. P., and Zilva, S. S., *Brit. J. Exp. Path.*, 1923, iv, 5.

[2] Cramer, W., Drew, A. H., and Mottram, J. C., *Proc. Roy. Soc. B.*, 1922, xciii, 449.

221 (2453)

A technique for the study of fat production in animals.

By WILLIAM E. ANDERSON* and LAFAYETTE B. MENDEL.

[*From the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

The study of the relation of food to the quality of fat laid down in the animal body is not new. The chief criticism, however, which applies to most of the past investigations concerns

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[3] The diluent used is that suggested by Rees, H. M., and Ecker, E. E., *J. Am. Med. Assn.*, 1923, lxxx, 621:

Sodium Citrate	3.8 gm.
Formaldehyde	0.2 cc.
Brilliant Cresyl Blue	0.1 gm.
Distilled water q. s. ad	100.0 cc.

* Fellow of Institute of American Meat Packers.

the number of variables which have entered into the experiments; particularly is this true in the experiments performed with farm animals and with mixed feeds of variable composition. We have approached the problem by means of the more recent methods of animal feeding and have adopted a standard basal ration in which protein and inorganic salts have been kept as constant as possible. After evisceration, the fat from the whole animal has been rendered in an autoclave kept under constant pressure for 14 or 15 hours. Our results have shown marked variations due to high carbohydrate feeding and the feeding of different oils. For example, with a preponderance of starch in the diet, a characteristic "hard" fat is obtained, while with the different oils typical "oily" fats are obtained. These "oily" fats give varying iodine numbers and refractive indices depending upon the oils fed. Solid fats are being fed in other diets. The conversion of an "oily" fat into a solid fat with high carbohydrate feeding is at present being studied.

222 (2454)

The alkaline isopotential point of the bacterial cell. Preliminary note.[1]

By C.-E. A. WINSLOW and H. J. SHAUGHNESSY.

[*From the Department of Public Health, Yale School of Medicine, New Haven, Conn.*]

Earlier studies on the electrophoretic migration of bacterial cells suggested the probable existence of a second isopotential point in the extreme alkaline range but results were irregular and uncertain.[2] Later work shows that such inconsistencies are due to a phenomenon observed in another connection,[3] the creation through the buffering power of the bacteria of zones of diminished alkalinity in their immediate vicinity. This effect can be eliminated by shaking the suspension vigorously for five minutes before placing it in the electrophoretic cell.

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[1] Studies here reported were aided by a grant from the Loomis Research Fund of the Yale School of Medicine.

[2] Winslow, C.-E. A., Falk, I. S., and Caulfield, M. F., *J. Gen. Physiol.*, 1923, vi, 177.

Observations made in this way, and with electrometric determination of pH,[4] show that there is a very definite alkaline isopotential point close to pH 13.5,—that for *B. cereus* lying at about 13.3 to 13.4 and that for *Bact. coli* at 13.6 to 13.8. Above this isopotential point the cells acquire a positive charge which increases rapidly to very high values with further increase in alkalinity.

223 (2455)

The buffer mechanism for the calcion concentration and the determination of calcion buffer values.

By I. NEWTON KUGELMASS.

[*From the Department of Pediatrics, Yale University, New Haven, Conn.*]

The calcion concentration of aqueous systems is regulated by calcion buffers. They are electrolytes which resist the change in calcion concentration upon addition or removal of calcium salts. The calcion concentration is stabilized in the presence of mixtures of weak acids and their salts which react to form insoluble normal calcium salts and soluble intermediate calcium salts.

The carbonates as calcium buffers may be expressed by the buffering reactions:

$$\left.\begin{array}{l} CaCl_2 \rightleftharpoons Ca^{++} \\ + \\ 2\ NaHCO_3 \rightleftharpoons 2\ HCO_3^- \end{array}\right\} \rightleftharpoons Ca(HCO_3)_2 \rightleftharpoons \left\{\begin{array}{c} CaCO_3 \\ + \\ H_2CO_3 \end{array}\right.$$

The phosphates as calcium buffers may be similarly expressed:

$$\left.\begin{array}{l} 4\ CaCl_2 \rightleftharpoons 4\ Ca^{++} \\ \\ 4\ Na_2HPO_4 \rightleftharpoons 4\ HPO_4 \end{array}\right\} \rightleftharpoons 4\ CaHPO_4 \rightleftharpoons \left\{\begin{array}{c} Ca_3\ (PO_4)_2 \\ + \\ Ca\ (H_2PO_4)_2 \end{array}\right.$$

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[3] Winslow, C.-E. A., and Falk, I. S., *J. Bact.*, 1923, viii, 215.

[4] Falk, I. S., and Shaughnessy, H. J., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 426.

The calcion concentration may be expressed in terms of calcion buffers by applying the law of mass action to the general form of the above buffering equations.

$$Ca^{++} = K \cdot \frac{\gamma_1 \, [HA]^n}{\gamma_2 \, [HA]^{2n}} = K^1 \frac{[HA]^n}{[HA]^{2n}}$$

Where [H A] and [B A] are the concentrations of the calcion buffering acid and salt respectively; γ_1 and γ_2 their activity coefficients at the calculated ionic strength; n is the ratio of the valence of calcium to that of the acid and K is an equilibrium constant. Expressed in logarithmic units,

$$\log \frac{1}{[Ca^{++}]} = pCa$$

$$\text{and } pCa = pK + n \log \frac{[B\,A]^2}{[H\,A]}$$

This expression enables the derivation of the calcion buffer value, ρ, the unit of which is the number of grain equivalents of calcium salt or acid necessary to change the calcion concentration one unit of pCa and is given by,

$$\rho = \frac{d\,[B\,A]}{d\ pCa} = \frac{2.3}{n} \, \frac{K'^{a}\,[H^+]\,[C]}{(K'^{a} + [H^+])(K'^{a} + 2[H^+])}$$

where n is unity for the carbonates and 2/3 for the phosphates.

The calcion buffer value expressed by equivalent equations shows that at any given hydrion concentration the calcion buffer value is directly proportional to the total concentration of buffer acid or salt. The calcion buffer value is independent of the nature of the weak acid provided it forms an insoluble normal calcium salt.

The calcion buffer value of the carbonates of normal blood is sum of the separate calcion buffer values.

The maximum calcion buffer value is attained when there are 0.586 parts of buffer salt and 0.414 parts of free buffer acid.

The molal calcion value at the maximum is given by the ratio 0.395/n where n is unity for the carbonates and 2/3 for the phosphates.

The pH at which the calcion buffer value is a maximum is given by

$$pH = pK'a + \log \sqrt{2}$$

which is pH 6.3 for the carbonates and pH 7.0 for the phosphates.

The molal calcion buffer value at pH 7.35 is 0.111 or 28 per cent of the maximum for the carbonates and 0.265 or 45 per cent of the maximum for the phosphates.

The calcion buffer value of the carbonates of normal blood serum at pH 7.35 is 3.5×10^{-3} and that of the serum phosphate is 0.5×10^{-3}. The combined calcion buffer value of blood serum is 4.0×10^{-3}.

224 (2456)

Preliminary report on human skin reactions to the "residue antigen" of the tubercle bacillus and to purified allied substances.

By E. S. GOODWIN and R. A. GUY.

[*From the Department of Pediatrics, Yale School of Medicine, New Haven, Conn.*]

Intracutaneous tests were made with solutions of the "residue antigen" prepared from dried tubercle bacilli, as described by Zinsser. This "antigen" shows none of the usual color reactions for the presence of protein. It has been shown by Zinsser and his co-workers to give specific precipitin tests with immune rabbit serum, and specific skin reactions of the tuberculin type in guinea pigs.[1]

Patients were tested with both O.T. and "residue antigen". We can conclude that the "residue antigen" from the tubercle bacillus is not injurious to the skin of the normal human being in amounts which cause injury to the skin of the individual allergic to the tubercle bacillus. This allergy of the infected individual is manifest as skin sensitiveness of varying degrees which parallels the sensitiveness to O.T.* We have no evidence of any correlation of the degree of sensitivity with the extent or activity of the infection.

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[1] Zinsser, H., and Barker, *J. Exp. Med.*, 1923, xxxvii, 275.

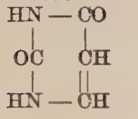
* Roughly 0.0007 mg. of "residue antigen" is equivalent to 0.01 of O.T.

No. of Cases	Reaction to O.T.		Reaction to "Residue Antigen"	
14 patients	0	10 mg.	0	0.07 mg.
2 patients	+ only to	1 mg.	+ only to	0.07 mg.
2 patients	+ only to	0.1 mg.	+ only to	0.007 mg.
4 patients	+ only to	0.01 mg.	+ only to	0.007 mg.
17 patients	+ only to	0.01 mg.	+ only to	0.007 mg.
3 patients	+ only to	0.01 mg.	+ only to	0.0007 mg.
1 patient	+ only to	0.001 mg.	+ only to	0.0007 mg.
11 patients	+ only to	0.001 mg.	+ only to	0.00007 mg.

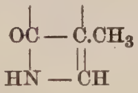
In the known tuberculous adults the reaction to a small amount of "residue antigen" was a sharp, well-defined area of injury without much swelling or redness, which was easily seen to be positive when measuring only 5 x 5 mm.

With the general plan of testing out the various non-protein derivatives of the tubercle bacillus as Professor T. B. Johnson could give them to us in pure form, we have made tests with a few nucleic acid derivatives which he had immediately available and very kindly gave to us. After preliminary toxicity tests in animals, intracutaneous injections of 20 mg. were made on five adults who showed marked positive reactions to 0.1 mg. of O.T. The following substances were used. There were no reactions.

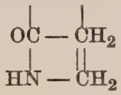
Uracil



Thymine



Hydro-uracil



The negative tests reported here are of interest only insofar as they indicate that these simple derivatives, if they were split off by the mechanism of the specific reaction, would not cause the type of injury under discussion.

225 (2457)

Further experiments on the antirachitic action of yolk of egg.

By ALFRED F. HESS and MILDRED WEINSTOCK.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

In previous communications[1] it was shown that egg yolk is able to confer protection or bring about cure in rickets, both in infants and in rats. Further experiments have shown that as little as one drop (0.05 gm.) a day (fed by pipette) is sufficient to protect rats which are on a ration markedly deficient in phosphorus. It is of much less protective value when the rats are given a rickets-producing dietary very low in calcium. Boiling the egg for twenty minutes does not appreciably diminish the potency of the yolk in relation to rickets. Drying the yolk and keeping it in a dried state markedly reduces its antirachitic value. When 0.05 gm. was given subcutaneously, the yolk did not confer protection, but this was accomplished by injections of 0.15 gm.

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[1] Hess, A. F., The therapeutic value of egg yolk in rickets, PROC. SOC. EXP. BIOL. AND MED., 1921, xx, 369; *J. Am. Med. Assn.*, 1923, lxxxi, 15.

Various fractions of egg yolk were tested in protective experiments on rats receiving the low phosphorus diet. It was found that the non-saponifiable fraction was protective and a preparation was obtained about 90 times as concentrated as the original yolk. It is, however, possible that its potency is not restricted entirely to this fraction.

226 (2458)

A study of hypersensitiveness to derivatives of hemolytic and non-hemolytic streptococci.

(Preliminary report).

By GEORGE M. MACKENZIE and FRANKLIN M. HANGER, JR.

[*From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*]

Hemolytic and non-hemolytic streptococci obtained from the interior of tonsils immediately after tonsillectomy have been used for the preparation of filtrates and extracts of intact organisms. A large number of patients with a variety of conditions has been tested by the intracutaneous method with these preparations.

The present report deals chiefly with certain reactions obtained with filtrates of young broth cultures. The intracutaneous injection of 0.01-0.04 cc. has produced in nearly all adults positive reactions which develop after a latent period of 8 to 12 hours. The intensity of the reaction has shown wide variation. The weaker reactions consist of a central, red, slightly tender elevation surrounded by slight swelling and erythema 1.5 to 2.5 cm. in diameter. Stronger reactions consist of a central pustule which upon culture has without exception proved to be sterile. Surrounding the pustule there is a red, hot, indurated zone, and outside of this a halo of erythema. The most intense reactions have measured 10 or 12 cm. in diameter and have been extremely tender. The full intensity of the reaction is attained after 24 to 48 hours. The reaction fades out in the course of two to ten days showing in the later stages a pigmented spot upon which, in some cases, a fine desquamation occurs. In a few instances there has been conspicuous lymphangitis extending up the arm from the site of the reaction with tender epitrochlear and axillary lymph nodes. The phenomena of a local infection with cellulitis, lymphangitis, lymphadenitis, have in the most intense reactions, been reproduced in a striking way. That these reactions are due to an acquired hypersensitiveness is shown by the fact that of 12 children under six months of age none has shown even a weakly positive reaction. Only one of 30 adults, tested with the same preparations, has failed to give a positive reaction.

An observation which may prove to be of significance was noted with a non-hemolytic streptococcus obtained from the excised tonsil of a rheumatic fever patient. A 24-hour filtrate prepared immediately after isolation of this organism has been found to be highly active. After cultivation for three weeks on blood agar repeated efforts to produce active filtrates from it have failed. The mechanism for the production of the active substance ceased to function after cultivation on laboratory media. A 48 hour filtrate from this organism prepared at the time that the active 24 hour filtrate was obtained, has proved to be entirely inactive. Forty-eight hour filtrates of the hemolytic streptococci studied have shown no less activity than the 24 hour filtrates; nor have we failed with the hemolytic strains to obtain active filtrates after cultivation on laboratory media for a period of three months. The active substance in the filtrates from both hemolytic and non-hemolytic streptococci is heat stable, withstanding boiling for ten minutes with little or no loss of activity.

Preparations kept in the icebox for three months have not deteriorated. Preliminary attempts to produce a neutralizing serum have yielded only equivocal results. Further work is in progress along this line. When a test is done on a skin area where a reaction has occurred, two or three weeks previously, it has been found that compared to a test done at the same time on a fresh site, the reinjection causes an accelerated reaction. The erythema and swelling appear in 3 to 5 hours, and reach their most intense development within 24 hours, and subside earlier than the reactions at a fresh site. There appears to be a certain lack of specificity in this accelerated response since the same phenomenon occurs if the reinjection is made with a filtrate of hemolytic streptococci at a site upon which the primary reaction was obtained with a non-hemolytic streptococcus filtrate. Four strains of non-hemolytic streptococci from human sources which have been grown on artificial media for several years, have produced 24 hour filtrates totally inactive on all except one of seven patients. So far the filtrates of non-hemolytic streptococci obtained from routine throat cultures have shown little or no capacity to produce skin reactions. Studies of the reactions with whole killed broth cultures and weak alkali extracts of whole organisms ground with powdered glass are in progress. It is already apparent, however, that some individuals react more strongly to these preparations than to filtrates, and in a few individuals the alkaline extracts free from constituents of the broth, have produced, not only the delayed reaction already described, but also the immediate wheal and erythema reaction which appears within thirty minutes and subsides after one to three hours.

We believe that the results thus far obtained justify the following conclusions: (1) hypersensitiveness to derivatives of hemolytic and non-hemolytic streptococci obtained from the throats or from the substance of tonsils, is demonstrable in a high percentage of adults. Very young children apparently do not react to this substance. (2) The non-hemolytic streptococci obtained from routine throat cultures in the absence of acute infection, have been found to be much less capable of producing active filtrates than non-hemolytic streptococci obtained from excised tonsils. (3) Certain non-hemolytic streptococci quickly lose the capacity to produce the active substance when cultivated outside the body. (4) The active substance present in 24 hour filtrates withstands boiling and does not rapidly deteriorate when kept at icebox temperature. (5) Reinjection at a site previously used for a reaction frequently reveals an alteration of the tissue response characterized by shorter latent period and earlier disappearance of the reaction. This alteration in the response is in some cases at least non-specific. (6) Some individuals react more strongly to filtrates, others to constituents of the bacterial cell.

227 (2459)

Observations of the nutritional effect of subcutaneous oil injections.

By B. SCHICK, MARGARET E. FRIES, JEROME KOHN, PHILIP COHEN.

[*From the Children's Clinic of Mount Sinai Hospital, New York City.*]

Experiments with subcutaneous oil injections have been frequently tried. The first clinical observations were published in 1869. Extensive animal experiments were carried out by Leube in 1895 and by Mills in Lusk's laboratory in 1911. Both considered oil injections were of practical value. Others have repeated this work using fat and oil for injections with varying degree of success. Winternitz was, for instance, unable to obtain good results.

We resorted to this method of treatment in a very serious case of Hirschsprung's disease (child 11 years of age). Following the total extirpation of the colon and sigmoid the child either refused or vomited most of his food. He also had loose stools. His condition was desperate, his weight having fallen from 63 to 47 pounds, a loss of 16 pounds.

The first two days we injected 50 cc. of sterile cotton seed oil. The child gained in weight, but after stopping the injections the weight remained stationary. For the following seven days we increased the amount of oil to 100 cc. per day. The general condition and appetite improved to such an extent that he took food by mouth better than before. His weight likewise increased.

Again the injections were discontinued, with the same effect upon his gaining, namely, almost no rise. We therefore decided to continue the oil injections for fifteen days more. Simultaneously the weight rose. As the child was eating well and his state of nutrition was much improved, we have discontinued the oil injections.

Apparently the greater part of the oil was absorbed, but there are a few areas of swelling undoubtedly due to the oil. Over a period of forty-one days thirty injections of oil were given. The total amount was 2250 gm., equal to 20,475 calories or 500 calories per day (750 nem). The child's weight rose from 47 to 58½ pounds, a gain of 11½ pounds.

In consequence of the good result obtained in this case, we tried subcutaneous injections of cottonseed and olive oil in fifteen infants and children suffering from chronic nutritional disturbances. These had a lowered tolerance for food given by mouth, but they seemed to utilize the oil given subcutaneously, as was demonstrated by gain in weight. 10 to 20 cc. were given daily or every other day to younger infants, while 20 to 30 and even 50 to 100 cc. were injected in older children.

The oil was absorbed more rapidly in the atrophic children than in those enjoying better nutrition.* It seems that the first injections of oil were better absorbed than the later ones. The best place for injections is probably the abdominal wall.

That the oil was absorbed at least to a certain extent was proven by fat determinations of the blood and autopsy examination in the case that died.

Work is now being undertaken on children and adults to determine whether such treatment with injection of oil is of real practical value and if so, which cases will respond best to this treatment. We will report later on our observations on the rate and ways of resorption of subcutaneous or intraperitoneal injected oil and fat, the effect on metabolism, the formation of lipolytic ferments, the effect on the blood picture, immune bodies lymphatic systems, etc.

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* As some children may be sensitive against cottonseed, olive oil can be used.

228 (2460)

The thyroid and specific dynamic action. Preliminary report.

By EMIL J. BAUMANN, with the technical assistance of LOUISE HUNT.

[*From the Laboratory Division of Montefiore Hospital, New York City.*]

The preponderance of experimental data on specific dynamic action of food in thyroid diseases, reported during the last decade by six investigators from as many laboratories, indicates that there is no change from the normal (DuBois,[1] Aub and Means,[2] Undeutsch,[3] Rolly,[4] Plaut,[5] and Liebesny.[6]) Weiss and Adler,[7] however, found an increased specific dynamic action in one case of Graves' disease, while in one case of myxedema it was also increased.

One never finds surviving cases of myxedema in infants or adults without active thyroid tissue. In Graves' disease thyroid activity while usually greater than normal, may be normal or subnormal. Since clinical conditions obtaining in thyroid diseases are so complex and variable, it seemed worth while to study the relation of thyroid to specific dynamic action of food under conditions more simplified and limited.

If the thyroid, and hence probably its secretion, were necessary for specific dynamic action to manifest itself then no specific dynamic action should result in completely thyroidectomized animals. If the thyroid gland is not a factor in this phenomenon, then there should not be much difference in the specific dynamic action before and after thyroidectomy.

Rabbits were chosen for the experimental animal because their thyroids can be completely removed without interfering with the inferior parathyroid glands. The greatest disadvantage of using an herbivorous animal for metabolism experiments in which a basal level is at times desired, lies in the fact that it is difficult to empty completely their alimentary canals, even after a four day fast, and yet have the animal otherwise in a physiological condition. By starving rabbits 36 to 48 hours their R Q's were usually below 74 which we regarded as satisfactory for our purpose. Under these conditions, the specific dynamic action of 25 gm. of glucose was found to average about 5 calories in six normal animals in the four hours following the administration of the sugar. The extreme variations were 2.4 and 7.4 calories although in the same animal closely agreeing results were obtained on different days, as a rule.

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[1] DuBois, E. F., *Arch. Int. Med.*, 1916, xvii, 915.

[2] Aub, J. C., and Means, J. H., *Arch. Int. Med.*, 1921, xxviii, 173.

[3] Undeutsch, quoted by Weiss and Adler, *Inaug. Dissertation*, Leipzig, 1913.

[4] Rolly, *Deut. med. Woch.*, 1921, xlviii^2, 887 and 917.

[5] Plaut, R., *Deut. Arch. klin. Med.*, 1922, cxxxix, 285.

[6] Liebesny, P., *Bioch. Z.*, 1924, cxliv, 308.

[7] Weiss, R., and Adler, E., *Klin. Woch.*, 1922, I, 1592.

The specific dynamic action of glucose was studied in six thyroidectomized animals, and in some of these it was determined before as well as after the thyroids were removed. In three of these animals the specific dynamic action gradually fell to zero. In the other three, after a drop in the extra heat produced, the specific dynamic action increased slightly. In each of these latter animals regenerated thyroid tissue was found at autopsy, while in the three animals which showed no specific dynamic action, no thyroid tissue could be found at autopsy.

The experiments, though limited in number, clearly indicate that the thyroid is an important factor in the development of the extra heat following ingestion of food. In the absence of thyroid secretion, there is no specific dynamic action, while in the presence of a small fragment of physiologically active thyroid tissue—less than 1/20 of the total amount of thyroid tissue of a normal animal—some specific dynamic action occurs.

The surgical work and necropsies were performed by Dr. David Marine. It is a pleasure to express my indebtedness to him for his aid.

229 (2461)

The production of lactic acid in diabetes following the administration of insulin.

By E. TOLSTOI, R. O. LOEBEL, S. Z. LEVINE, and H. B. RICHARDSON.

[*From the Russell Sage Institute of Pathology in Affiliation with the Second Medical (Cornell) Division of Bellevue Hospital, New York City.*]

It is evident from recent work on normal animals that following the administration of insulin in large doses marked changes occur in the glucose, lactic acid,[1] and inorganic phosphate[2] content of the blood. Since these constituents seem to be intimately concerned with the metabolism of carbohydrate, it was desirable to extend the study of them to human beings, more especially to diabetics. Also, since the combustion of glucose as well as the formation of lactic acid has a profound influence on the respiratory quotient the metabolism was determined using the respiration calorimeter of the Russell Sage Institute of Pathology.

Four patients with uncomplicated diabetes were studied. They varied from extremely mild to moderately severe. In each case the effect of a single intravenous dose varying from 8 to 33 units of insulin (Iletin, Lilly) was observed. The blood was taken before, and between 1 and 2 hours after, the injection, and analyzed for sugar, lactic acid, and inorganic phosphates. The respiratory metabolism was determined in the calorimeter beginning about 15 to 75 minutes after the injection and continuing for 45 minutes. The patient was then removed from the calorimeter, and a blood sample was drawn for analysis. Immediately after, he was returned to the calorimeter and observed for another three hours. For comparison, a three-hour basal observation of the previous day was used.

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[1] Briggs, A. P., Koechig, I., Doisy, E. A., and Weber, C. J., *J. Biol Chem.*, 1924, lviii, 721.

[2] Wigglesworth, V. B., Woodrow, C. E., Smith, W., Winter, L. B., *J. Physiol.*, 1923, lvii, 447.

TABLE I.

Name	Time	Glucose	Lactic acid	Inorganic PO_4	R. Q.	Units of insulin	Carbohydrate metabolized	Remarks
		mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.			per hr. gm.	
E. V.	Before	362	22.4	2.4	0.745		0.98	Severe diabetic. Total G tolerance 40 gm.
	1 hr. 20 m.					33		
	After	148	21.0	2.5	0.799		3.06	
	Before	292	27.3	2.4	0.738		0.69	
	1 hr. 30 m.					15		
	After	210	23.1	1.88	0.809		3.67	
	Before	182	24.5	3.4				No respiration studies.
	1 hr.					20		
	After	64.5	49.0	2.0				
J. D.	Before	108	14.0	3.5	0.836		5.61	Mild diabetic. Total G tolerance 208 gm. Profuse perspiration. Tem. dropped .7 deg. in 1st 45 m., pulse rose 60-96. Fell asleep.
	2 hr. 20 m.					15		
	After	70	24.5	3.0	0.954		9.70	
L. M.	Before	154	15.7	3.0	0.804		4.14	Mild diabetic. Total G tolerance over 200 gm.
	1 hr. 15 m.					10		
	After	94	45.7	1.7	0.920		10.14	
J. M.	Before	125	16.8	2.7	0.792		5.28	
	1 hr. 30 m.					8		
	After	111	21.0	2.8	0.825		7.19	

The results in the table clearly demonstrate that the invariable fall of blood glucose following insulin administration is not always accompanied by a rise in lactic acid.

Three separate observations were made on patient E. V. In only one instance was the lactic acid concentration increased after insulin. Although the absolute drop in blood sugar was greater in the other two, 214 mg., and 82 mg. per 100 cc. respectively, no rise in the lactic acid was found.

With the patients J. D. and L. M. the blood glucose fell 38 mg. and 60 mg. per 100 cc., yet the rise in the lactic acid was considerable, while in patient J. M. the fall of the blood glucose noted was small and no change in the lactic acid was observed.

These data justify the conclusion that the production of lactic acid following the administration of insulin depends less upon the absolute fall in the blood sugar than upon the level finally reached.

It is also apparent that insulin may cause a rise of the respiratory quotient without producing any increase in the lactic acid concentration. This rise can, therefore, be ascribed to the stimulating effect on the oxidation of carbohydrate.

If the production of lactic acid were the direct result of the fall in blood sugar it would *not* be expected with a rise. Data were obtained on this point by means of adrenalin.

Four non-diabetic subjects free from any cardiac or hepatic involvement were studied. After lunch 15 to 25 minims of adrenalin were administered subcutaneously. Blood samples were drawn before and exactly 1 hour after the adrenalin injection and analyzed for glucose, inorganic phosphates and lactic acid. The respiratory metabolism was not studied.

From Table II it will be seen that whenever the adrenalin produces a rise of blood sugar there occurred a concomitant lowering of the inorganic phosphates, as well as a rise in the lactic acid.

Discussion.

These results do not favor the theory that insulin acts by converting glucose into lactic acid. Although it causes a rise in the lactic acid the effect is far from constant. Moreover, no quantitative relation is apparent between the blood sugar and the production of lactic acid, such as the theory of Briggs and his associates would demand. With insulin a sharp *fall* in blood *sugar* can be produced *without* an increase in lactic acid. With

TABLE II.

Case	Time	Glucose	Lactic acid	Inorganic P	Remarks
		mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	
1	Before	127	14.	2.6	Adrenalin MXV
	1 hr.				
	After	235	31.5	2.0	
	Before	100	16.1	2.6	
	1 hr.				Adrenalin MXV
	After	170	26.6	1.76	
2	Before	125	19.6	2.7	
	1 hr.				Adrenalin MXX
	After	250	37.1	1.9	same patient
3	Before	104	15.4	3.1	
	1 hr.				Adrenalin MXV
	After	230	21.0	1.5	
4	Before	112	19.0	2.4	
	10 m.				Adrenalin MXV
	After	117	26.6	2.4	

adrenalin a marked *rise* can occur, and instead of a *fall* in lactic acid we observed an *actual rise* amounting often to 100 per cent. The results suggest that there is some factor common to the action both of insulin and adrenalin, which increases the lactic acid in spite of the divergent effects on the blood sugar. Such a common denominator might be a local asphyxia which is a well known cause of the production of lactic acid.

A comparison of the effects of insulin and the adrenalin leads to the following conclusions:

(1) Both extracts lower the inorganic phosphate of the blood.

(2) Extreme drops in blood sugar may occur with no change in the lactic acid concentration. With our patients increases in lactic acid were observed *only* when an insulin hypoglycemia was produced and this may turn out to be the general rule.

(3) Lactic acid increases with an adrenalin hyperglycemia.

(4) Insulin causes an increase in the respiratory quotient over and above that which can be accounted for by the production of lactic acid. This is evidence of the stimulating action exerted by the extract on the oxidation of carbohydrate.

230 (2462)

Zone phenomena in the Kahn precipitation test for syphilis.

By ELIZABETH W. ROCKSTRAW and J. G. HOPKINS.

[*From the Department of Bacteriology and the Department of Syphilis and Dermatology, Columbia University, New York City.*]

The precipitation test for syphilis described by Kahn[1] is carried out with three tubes containing equal amounts of serum and diminishing amounts of antigen.

Three types of positive test are observed: In one, a strong precipitation occurs in the first tube, and progressively weaker reactions in the second and third; in the second type of positive test an equally strong precipitation occurs in all three tubes; in the third type, the precipitation is strongest in the third tube, and weaker in the second and first.

Zone phenomena in the Wassermann reaction were described in 1912 by Coca and L'Esperance[2] and more recently by Kahn and Johnson.[3] We have not observed zones similar to those described by these authors in which the strength of fixation increased as the antigen was diluted. We have, however, observed that very strongly positive sera when progressively diluted fixed with smaller amounts of antigen up to a certain maximum and in still higher dilution only with concentrated antigen.

The significance of such zone phenomena in precipitation and complement fixation reactions was carefully studied by Gay[4] and Dean.[5] Their findings have been confirmed by Parker[6] and by others who have studied the question. Dean pointed out that as a serum is diluted it is necessary to dilute the antigen correspondingly in order to obtain maximum fixation or precipitation and that consequently a serum which fixed or precipitated only with concentrated antigen, as a rule, had a higher antibody content than one which fixed with highly diluted antigen.

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[1] Kahn, R. L., *J. Am. Med. Assn.*, 1923, lxxxi, 88.

[2] Coca, A. F., and L'Esperance, E., *Ztschr. f. Immunitäts., Orig.*, 1912, xiv, 139.

[3] Kahn, R. L., and Johnson, S. R., *J. Infect. Dis.*, 1922, xxxi, 416.

[4] Gay, F. P., Univ. of Calif., Publications in Path, 1911, ii, 1.

[5] Dean, H. R., *Z. Immunitäts., Orig.*, 1912, xiii, 84.

[6] Parker, J. T., *J. Immunol.*, 1923, viii, 22.

TABLE I.
Fixation zone in Wassermann test.
Serum from untreated case of secondary syphilis.

Antigen 0.1 cc. dilution	Serum 0.05 cc.		Serum 0.02 cc.							No serum Control
	Undiluted	Undiluted	1—2	1—5	1—10	1—20	1—50	1—100	1—500	
1—10	++++	++++	++++	++++	++++	++++	++++	+++	0	0
1—20	++++	++++	++++	++++	++++	++++	+++	0	0	0
1—50	++++	++++	++++	++++	++++	++++	+++	0	0	0
1—100	+	++++	++++	++++	++++	++++	++	0	0	0
1—200	0	+++	++++	++++	++++	++++	+	0	0	0
1—500	0	0	++	++++	++++	+++	0	0	0	0
1—1000	0	0	0	0	+	+	0	0	0	
1—2000	0	0	0	0	0	0	0	0	0	
No antigen (control)	0	0	0	0	0	0	0	0	0	

TABLE II.
Precipitation zone in Kahn test.
Serum from untreated case of secondary syphilis.

Serum 0.3 cc.		Serum 0.15 cc.					
Antigen 0.3 cc. Dilution	Undiluted	Undiluted	1—2	1—5	1—10	1—20	1—50
1—1	++++	++++	++++	++++	++++	+++	0
1—2	++++	++++	++++	++++	++++	++++	0
1—5	+++	++++	++++	++++	++++	++++	++
1—10	+++	++++	++++	++++	++++	++++	+++
1—20	0	+++	++++	++++	++++	++++	+++
1—40	0	0	0	+	+++	+++	++

That the phenomena observed in the Kahn test follow this rule is shown by experiments such as that given in Table II, in which increasing dilutions of serum are tested with increasing dilutions of antigen. If one compares the first five results in column 1 with the corresponding results in column 4, it will be seen that by diluting the serum one can convert a reaction strongly positive in the first tube and negative in the last to one that is strongly positive throughout. Observing the results in the seventh column, it is seen that further increasing the dilution of serum converts the result to one negative in the first tube and strongly positive in the last. In other words, that the first type of reaction indicates a strongly reactive serum, the last type a weakly reactive serum.

The same result is arrived at if we take the strength of the cholesterin Wassermann test as an index of the strength of the serum.

TABLE III.
Correlation of Kahn and Wassermann reactions.
(Figures indicate number of sera giving each combination of results.)

Kahn Reaction			Wassermann Reaction (Cholesterol Antigen).				
1st tube	3rd tube	Negative	+	++	+++	++++	Average Wasserman val.
++++	0					11	4.0
+++	0					2	4.0
++	0					1	4.0
++++	+		1		1	12	3.7
++	+					1	4.0
++++	++				3	14	3.8
+++	++		1		1	2	3.0
++++	+++		1	2	3	14	3.5
++++	++++	3	4	1	26	87	3.5
+++	+++		1			2	3.0
++	++			1	1	3	3.4
+	+		1	1			1.5
+++	++++	2	4		4	8	2.8
++	++++	2	3		6	2	2.2
++	+++	1	2		2		1.6
+	++++	2	4		3		1.3
+	+++	1	2			1	1.5
+	++	1	2		1	1	1.8
0	++++	2	5		1		1.0
0	+++	4	7	2	1		1.0
0	++	17	8			1	0.46
0	+	21	4		1		0.27
0	0	667	7		1		0.015

In Table III are shown the Wassermann results obtained with 1,000 consecutive specimens, giving varying results with the Kahn test. The figures indicate the number of sera giving each combination of reaction. It will be seen that those in which a Kahn reaction of the first type (stronger in the first tube than in the last) was obtained, the average Wassermann value was 3+ to 4+; those giving the second type of Kahn test (equally strong in all three tubes) the average Wassermann value was 1.5+ to 3.5+; that in those giving the third type of Kahn test (stronger in the last tube than in the first) the mean Wassermann value was 0.3+ to 2.8+. To further study this point we titrated out the strength of the Wassermann in the number of sera giving a ++++ reaction and found that the very strong reactions were obtained only in those showing the first type of Kahn reaction.

These experiments are reported because they illustrate so well the principles laid down by Dean and because they are of some practical importance in interpreting the precipitation test. A true idea of the strength of the reaction cannot be obtained by averaging the values of the three tubes. The first type reaction described should be regarded as strongly positive, the third type as weakly positive, and the second as of intermediate strength.

231 (2463)

Tbe basal metabolism in vitamin B deficiency.

By HARRY J. DEUEL, JR., and ROBERT WEISS* (Prague).

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

The heat production in dogs during rest has been determined with the respiration calorimeter when these animals were fed with diets deficient in vitamin B. The purified food mixture fed to Dogs XXIV and XXV was complete in all respects with the exception of vitamins B and C. It was composed of casein, butter fat, lard, sucrose, salt mixture, and bone ash. Vitamin C was omitted since it has been shown that dogs, when fed with such mixtures containing vitamin B, remain in normal condition over long periods of time and show no effects from this lack.[1] Basal metabolism measurements were made by the usual technique employed in this laboratory.

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* Medical Fellow of the Rockefeller Foundation.

On Dog XXIV the metabolism was determined at approximately weekly intervals during the course of the ingestion of the standard vitamin B-free diet. In this animal the basal heat production gradually fell, but the diminution was no greater than that which would be expected from the decreased body weight of the animal due to a decreasing food intake. On the day before the development of polyneuritic symptoms and on the four days following the removal of these symptoms by the administration of 20 gm. of yeast vitamin (Harris), the basal metabolism was remarkably constant, thus indicating that vitamin B deficiency *per se* does not influence heat production.

In the case of Dog XXV the basal metabolism was determined at the height of polyneuritis and was found to be about 25 per cent higher than the normal value to which it gradually fell after the removal of the symptoms. In this case the high heat production must have been due to the extreme muscular tonicity caused by the polyneuritic condition and only indirectly to the vitamin B deficiency.

That vitamin B does not influence the metabolism of the normal dog when it is given in an amount greater than that necessary for maintenance is shown in the case of Dog XIX.[2] This animal, which was usually given a diet containing a small but sufficient amount of vitamin B, did not show an altered basal metabolism following the augmentation of this supply by the daily addition, over a period of several months, of doses of 1 gm. of vitamin B concentrate to the same diet that this animal had previously received.

The basal nitrogen elimination—by which we mean the endogenous metabolism 16 to 18 hours or more after food—was found to be three times as high during polyneuritis as that after the recovery following the administration of vitamin B. During the height of polyneuritis in Dog XXV this value was found to be 0.273 gm. per hour, while 10 days later, after recovery, it had gradually fallen to 0.095 gm. hourly. After 6 days of fasting, at which time the animal had attained the same body weight as that which prevailed during polyneuritis, the basal nitrogen output amounted to 0.095 gm., thus indicating that the high nitrogen excretion during polyneuritis is not attributable to the starvation at this time. Subsequent investigations now in progress on the same animal seem to indicate a relationship between the basal nitrogen content of the urine and the extent of vitamin B starvation.

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[1] Cowgill, G. R., Deuel, H. J., Jr., and Smith, A. H., *J. Biol. Chem.*, 1924, lix, 11.

[2] These data were kindly supplied us by Dr. D. Rapport.

It may be concluded that vitamin B starvation *per se* does not alter the basal metabolism, although when complicated with polyneuritis an increased metabolism is evident; an increased nitrogen catabolism is also found in the latter condition, as shown by urinary nitrogen excretion.

232 (**2464**)

Studies on the chemistry of cod liver oil. II. A cod liver oil concentrate manifesting both antirachitic and antiophthalmic properties.*

By HARRY E. DUBIN and CASIMIR FUNK.

[*From the Biochemical Department of the H. A. Metz Research Laboratories, and the Biochemical Laboratory, College of Physicians and Surgeons, Columbia University, New York City.*]

In recent years, the demonstration of the specificity of cod liver oil for rickets has provided a stimulus for further attempts to isolate and identify the active principle of the oil. Though the goal has not been reached, nevertheless appreciable progress has been reported by various investigators.

The problem has been approached by devious routes, but the most promising finding has been the demonstration that the vitamines known to be present in cod liver oil—the antirachitic and antiophthalmic—are to be found in the ether-soluble, non-saponifiable portion of the oil.

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* This investigation was started in January, 1922, and is still being continued.

On saponifying cod liver oil, Steenbock, Nelson and Hart[1] obtained a product that was curative for both rickets and ophthalmia. Using an analogous process, Zucker, Pappenheimer and Barnett[2] and also Takahashi[3] reported results somewhat like those of Steenbock, Nelson and Hart. Some time later, Zucker[4] extracted cod liver oil with alcohol and then saponified the alcoholic extract. He thus obtained a product which contained only the antirachitic vitamine, the antiophthalmic vitamine having been destroyed in the procedure.

By means of a special extraction and saponification process we have prepared from cod liver oil a concentrate manifesting both antirachitic and antiophthalmic activity. The residual cod liver oil is entirely free from vitamines.

The crude concentrate—0.5 gm. from 1000 gm. cod liver oil—is obtained as a brown syrupy mass which, on standing, crystallizes in light yellowish brown needle-like crystals radiating from a central point.

This highly concentrated substance, which is insoluble in water but freely soluble in the ordinary organic solvents, contains practically all of the antirachitic and antiophthalmic vitamines originally present in the fresh cod liver oil.

In other words, if this 0.5 gm. vitamine concentrate is incorporated in 1000 gm. cottonseed oil, the resultant product is practically equivalent in vitamine activity to the original cod liver oil. The same is true if this 0.5 gm. concentrate is incorporated in 1000 gm. of an inactive base such as sugar.

On eliminating cholesterol and other inert substances, the above 0.5 gm. concentrate is reduced to 0.1 gm.—a concentration of 1 in 10,000. By working with extreme care, it is possible to obtain an active product showing a concentration of 1 in 15,000.

Thus far we have been unable to establish the exact chemical composition of this highly concentrated vitamine substance since it is undoubtedly still far removed from the actual chemical entity.

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[1] Steenbock, Nelson and Hart, *Am. J. Physiol.*, 1921-1922, lviii, 14.

[2] Zucker, Pappenheimer and Barnett, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 167.

[3] Takahashi, *Proc. Jap. Chem. Soc.*, *J. Chem. Soc.* (Japan), 1922, xliii, 828.

[4] Zucker, PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 136.

However, the presence of C, H, O and S have been demonstrated. N and P are absent, as are also the halogens. The H_2SO_4 reaction is obtained just as with fresh cod liver oil, showing that the vitamine activity of the concentrate has been retained. Further, it is not toxic, even in large doses.

The advantages of such a concentrate are twofold. First, it provides a starting material for chemical work directed to the actual isolation and identification of the vitamines present in cod liver oil. Second, it provides an effective method of carrying out cod liver oil therapy, whether experimental or practical.

Experiments with over 300 rats kept upon a variety of vitamine-free and rickets-producing diets have shown that the cod liver oil concentrate described above is capable of both curing and preventing rickets and ophthalmia. The concentrate was administered apart from the diet, mixed either with an inactive oil or with sugar. The results were controlled by x-ray of the tibia and by microscopic examination of rib sections.

Clinical experiments[5] have revealed ample evidence that the concentrate is equally effective in children. In this work, the concentrate was mixed with sugar and administered in the form of 1 grain tablets, arbitrarily prepared to be the equivalent of half a teaspoon of cod liver oil. Due to the high degree of concentration, it is of course possible to prepare a small tablet containing as much or as little concentrate as may be desired for the particular experiment at hand.

It is our belief that infants, as early as one month after birth, should be given cod liver oil, or its equivalent in the form of a concentrate, as a prophylactic against rickets and other nutritive disturbances traceable to the lack of fat-soluble vitamines in the diet.

Detailed data of the above work will be published shortly.

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[5] Conducted by Dr. Louis Fischer during the past year at The Infantorium, Heckscher Foundation Building, New York City. These results are reported in the next paper.

233 (2465)

Clinical results in cases of rickets treated with an active concentrate prepared from cod liver oil.

By LOUIS FISCHER.*

[*From the Infantorium and Nursery of the Heckscher Foundation, New York City.*]

The clinical observations extend over a period of more than one year. All cases were infants and children of the pre-school age. In the series under investigation 37 cases had rickets in a mild or severe form. The symptoms were confined to the head, thorax and the extremities. No case was diagnosed as rickets until the Roentgenologist called the case rickets. Our studies were continued and supervised by means of the x-ray. This showed where there was or where there was not improvement. Increase in weight and growth were symptoms that were stressed and metabolism was regarded as the most important result in a successful case. The blood examination was made in some cases and this was studied to determine an improvement if any existed.

Very many cases had eczema associated with rickets. In all, over 900 infants were examined and in the series 37 had backwardness in dentition, inability to walk or stand and rachitic manifestations confined to the bony system.

Constipation seemed present in 50 per cent of the cases. The other half had catarrhal, greenish and foul smelling stools. Atony of the gastrointestinal canal noted by loss of appetite, constipation or loose bowels was present in most cases. The urine showed acetone and traces of diacetic acid and indican.

In eight of the series an infection of the respiratory tract resulted in a bronchopneumonia and death.

The susceptibility of rachitic cases to the acute catarrhal diseases impressed us with the importance of avoiding unnecessary exposure at night and in the damp weather during our attempt to harden our infants and render them less susceptible to cold drafts and moisture. This susceptibility to colds and coughs and catarrhal infections seemed lessened after the first week, or two weeks at the most, of treatment with cod liver oil concentrate.

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* Introduced by Harry E. Dubin.

In the series here presented, we were greatly impressed with the rapidity in which improvement was found. The cases were given the best hygienic supervision and the food was modified in quantity as well as in quality and cod liver oil concentrate was added as the vitamine and reconstructive factor. Progress was always noted, but it was slow progress.

Our observations were confined to two classes of cases. In the first series we depended on food, fresh air and cod liver oil, and in the second series, food, fresh air and sunshine. The influence of the sunshine was notable not only on the weight, sleep, metabolism and on the blood cells, but it also showed a marked healing tendency and influence on the calcium deposit in the bones. The influence of sunshine was apparent in every case. We have an excellent roof garden on which continuous sunshine is found.

The x-ray reports showed considerable difference in the healing process and the calcium deposit. Improvement was usual after two weeks of steady treatment and healed rickets was reported in most cases after one month's continuous treatment.

In the series of clinical observations here reported, we employed a cod liver oil concentrate similar to that used by Dr. Dubin in his studies on experimental rickets. His results are reported elsewhere in this issue.

The cod liver oil concentrate, as first employed by me, was mixed with syrup. Subsequently, it was found more convenient to give a 1 grain tablet in which the cod liver oil concentrate is mixed with sugar, each tablet being equivalent to a half teaspoon of fresh cod liver oil.

234 (2466)

The biological action of the beta rays of radium.

By ISAAC LEVIN.

[*From the Cancer Division of the Montefiore Hospital, New York City.*]

Methods of radium therapy commonly employed consist in the application of the radium over the surface of the area to be treated or in the insertion into the tissue to be influenced of metal needles containing radium. In both instances the radium rays are filtered in such a manner that only the gamma rays penetrate into the tissues and act as a therapeutic agent.

More recently a method of radium therapy has been developed by the aid of which not only the gamma but also the beta rays of radium are employed in therapy. This method consists in the following:

Radium emanation—an elementary body in the state of a heavy gas—is the first active product of decomposition of radium. It is collected by means of appropriate apparatus in capillary glass tubes from 1 to 5 mm. long and 0.25 mm. in diameter. These tubes may be made to contain anywhere between 0.1 to several millicuries of radium emanation. They are inserted into the tissue to be influenced by the aid of a trocar. The thin glass wall of the capillaries filters off only the alpha rays and allows the free passage of both the beta and gamma rays.

It is difficult to separate the beta rays from the gamma rays and study the biological action of the former, though R. Abbe made recently an attempt in the direction. However, the biological and therapeutic results obtained by the action of gamma rays alone and by the combined action of beta and gamma rays differ to such an extent that certain conclusions as to the action of the beta rays can be drawn.

In coöperation with Michael Levine, the writer employed this method experimentally on normal and neoplastic tissues of animals and plants. The capillary glass tubes were inserted into normal liver, spleen and bone marrow of rabbits, into muscles and testicles of rats and mice, into sarcoma of rats, carcinoma of mice, into normal young plant tissue, into crown galls of geranium and into club roots of cabbage.

A gross and microscopical analysis of the irradiated tissues was made. The zone of tissue immediately surrounding the glass capillary was in a state of complete necrosis. The second zone showed characteristic gamma ray irradiation changes in the protoplasm and nuclei of the cells. This latter zone was more extensive in tumor than in normal tissues.

Alongside of the experimental investigations the writer subjected to microscopical study tissues obtained on clinical material at various intervals after the insertion of the glass capillaries. In general the clinical results are very significant. Pathological conditions, which do not yield to large quantities of filtered rays, respond readily to the beta ray action.

Microscopically human tissues examined one to two weeks after the insertion of the capillaries show the same condition as in animal and plant tissues, *i. e.,* a narrow zone of necrotic tissue and a second larger zone which shows the effect of gamma radiation. In material studied six to eight weeks after the insertion of the capillaries the necrotic tissue is found mostly to be absorbed and the second zone of tissue is replaced by connective tissue.

A superficial analysis of the results obtained may produce the impression that the beta ray which acts on the first zone of tissue is a simple caustic agent. A closer study of the phenomena involved, however, shows that the mechanism of the action of beta rays differs widely from the caustic effects of heat or chemical agents and is qualitatively analogous to the action of the gamma rays.

The biological action of the gamma rays of radium and of the analogous x-rays must depend in the ultimate analysis of the intraatomic action of these rays on the atoms of the elements. This action in accordance with the present day conception of physics is as follows:

Gamma rays and x-rays are rays of light of very small wave lengths. When they enter an atom of matter they disturb the electronic equilibrium and free a certain number of electrons of the influence of the positive nucleus of the atom. These free electrons travel in a certain direction with a given velocity and as a result produce within the ether secondary scattered or characteristic x-rays. The results will be the same whether the original agents were the gamma rays or the x-rays.

A biological effect of the rays on tissues means a change in the structure of the tissues and must be a result of changes in the atoms of the tissues. A gamma ray entering the tissues sets into motion a number of electrons. The latter produce secondary x-rays which influence the other component parts of the tissue. Beta rays are themselves electrons in motion and when they enter the tissue they must produce analogous secondary x-rays because beta rays of radium are products of the gamma rays of radium and must have a velocity similar to the velocity of the electrons produced in the tissue by the gamma rays of radium. Consequently the biological action of the gamma and beta rays must be analogous. The difference is quantitative and is due to the fact that the ratio of beta and gamma rays in a unit of radium is about 100 to 1. Five millicuries of radium emanation distributed in 10 capillaries will destroy 10 cubic millimeters of carcinoma. To produce the same effect by surface application the gamma rays of 500 millicuries would have to be employed. The statement made by physicists that the biological effect of one millicurie of radium emanation buried in the tissues equals 132 millicurie hours of surface application of filtered radium must be corrected since it does not take into account the action of the beta rays and their secondary x-rays.

235 (2467)

A crystalline substance from the parathyroid glands that influences the calcium content of the blood.

By LOUIS BERMAN.*

[*From the Laboratory of Biological Chemistry, School of Medicine, Columbia University, New York City.*]

Parathyroid glands from oxen were extracted with acidified alcohol. Lipins and proteins were removed from the extract. Crystalline material was obtained by concentration which increases definitely the calcium content of the blood when dissolved in Ringer solution and injected into the circulation. Its effects upon the calcium content of the blood have been compared with those of adrenalin, pituitrin and insulin. Whether this crystalline material is the active calcium-mobilizing substance in the gland or merely a carrier of it in the preparation, remains to be determined. Its separation is additional evidence in favor of the view that the parathyroid glands secrete a hormone that influences the calcium content of the blood.

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* Introduced by William J. Gies.

236 (2468)

Phenomena of blood coagulation with special reference to a transplanted tumor of the rabbit.

By C. M. VAN ALLEN.*

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

A certain proportion of rabbits, about 20 per cent, inoculated with the tumor now employed in our work[1] develop a fulminating malignant disease and die with widespread metatases as early as 3 to 5 weeks after inoculation. When these animals are examined at autopsy it is frequently noticed that the blood retained in the heart or in contact with raw surfaces may show little or no tendency to the formation of a firm clot. The contrast between this behavior and the apparently normal formation of blood clots in rabbits of the same group, which develop a more benign form of disease, led to the following quantitative study of the phenomena of blood coagulation in tumor bearing rabbits as a possible factor of importance in determining the course of malignant disease.

It was deemed advisable in beginning this investigation, to approach the problem first from the standpoint of the gross prenomenon of coagulation, separating this into two components, clotting time and clot contractibility. In the measurement of the so-called clotting time, the many methods thus far elaborated take as an endpoint the attainment of a certain consistency of clot in its resistance to one force or another. This necessarily confuses the idea of time *per se* of the initiation of coagulation with that of the firmness or contractibility of the clot and rules out all such methods for the present purpose. It was necessary, therefore, to devise methods and instruments for the investigation of this problem. The details of the methods employed and the description of the instruments used will be reported elsewhere.

For the present, it is sufficient to say that an instrument was devised which made it possible to observe the earliest gross precipitation of fibrin in a thin film of blood. Clotting time determined in this way is expressed in seconds, and the normal for the rabbit lies between 20 and 30 seconds. Under abnormal conditions the time may be diminished to 8 or 10 seconds, or extended as long as 2 or 3 minutes.

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* Introduced by Wade H. Brown.

[1] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, xxxvii, 601, 631, 799, 811, *ibid.*, xxxviii, 347, 367, 385.

For the accurate estimation of clot contractibility it was also necessary to elaborate a procedure, which employs as an index of contractibility the amount of serum expressed from the clot under uniform conditions. The values of serum expressed from the blood clot are translated into terms of percentage of the quantity of plasma present in the whole blood. Measurement of the serum expressed from the clot is made at the end of 1 hour, in order to indicate the rate of contraction of the clot; and again at the end of 6 hours, in order to ascertain the final completeness of contraction. The normal 1 hour expression of serum in the rabbit lies between 82 per cent and 88 per cent of the available plasma, and the final (6 hour) expression between 85 per cent and 90 per cent.

Clotting time and clot contractibility, estimated in this way, have been followed consecutively in normal rabbits, and it has been found that, provided the tests are made in the fasting animal, the results are remarkably constant from day to day. The equilibrium of these phenomena is upset immediately by the advent of any of the spontaneous diseases to which rabbits are prone, such as snuffles, pneumonia, mastoiditis, subcutaneous abscess, and even infestation with vermin.

These gross phenomena of blood coagulation have been investigated systematically in 20 tumor rabbits at intervals of 6 or 8 days from the date of inoculation until the death of the animal or up to the expiration of 2 months. At the same time careful clinical observations were made, including the rate of growth and character of the primary tumor, the occurrence of metastases, and the general physical condition of the animal. At the time of death or at the end of the 2 months period of observation autopsy was performed in detail.

Considered broadly, the clotting time, the rate of clot contraction, and the completeness of clot contraction, behaved in a similar fashion, and for the present purposes the three phenomena are referred to collectively as the efficiency of coagulation. However, there was frequently exhibited a clear-cut individualism in these values which deserves notice, a tendency displayed by one or another to compensate for failure in the others by assuming an abnormally high value.

It may be stated, in general, that the efficiency of coagulation was found to run parallel with the fluctuations in resistance manifested by the animal against the tumor. With the first local growth and invasion by the primary tumor and at a time when no other evidence of reaction on the part of the host could be recognized, definite alterations were apparent in the blood clotting mechanism. These alterations took various forms, in some of the rabbits there was a sharp rise in the efficiency of clotting time and clot contraction, and in others a fall in the efficiency of these functions. As the disease progressed, a few of the former group maintained the initial hyper-efficiency of coagulation throughout, and it was these animals that recovered completely from the effects of the tumor. On the other hand, a few of the latter group maintained the initial hypo-efficiency of coagulation, and it was these animals, without exception, that died at an early period with the most extreme form of malignancy. The remaining animals of each group did not persist in the initial type of response, but subsequently went through a series of elevations and depressions in clotting efficiency. The depressions that occurred were frequently associated with loss of body weight and strength, and were almost invariably followed in a few days by the appearance of a fresh crop of clinically demonstrable metastases. On the other hand, the occurrence of increased efficiency in coagulation was accompanied by a return of weight and vigor and by a cessation of metastatic deposit. Moreover, those animals which exhibited frequent and extensive depressions in efficiency of coagulation were, in almost every case, ones which developed many metastases and ultimately succumbed to the disease; while those which displayed infrequent and inconsequential depressions usually recovered.

There was one notable exception to this general plan of behavior which deserves emphasis. Occasionally rabbits were found which maintained a fairly high level of clotting efficiency throughout the course of the disease, but, nevertheless, gave way to extensive primary and metastatic tumor involvement. When examined at autopsy, the metastasis was found to be almost entirely of a type suggesting a lymphatic distribution of tumor cells, in that those organs not directly in the path of the regional lymphatics were untouched. This picture contrasted strikingly with that presented by rabbits, which during the course of the disease displayed the most marked inefficiency of coagulation, for in these the great mass of metastatic deposit was lodged in parts of the body to which it very evidently was carried by the blood stream. On the strength of these extreme examples, a more critical analysis of the other cases brings to light the fact that metastases appearing shortly after a depression in coagulability were chiefly of a blood borne type.

From the work done thus far, it may be concluded that (1) certain characteristics of the blood clotting phenomenon in rabbits, *viz.*, the clotting time and the rate and completeness of clot contractibility, remain remarkably constant in animals free from disease; (2) that alterations in these phenomena occur in rabbits suffering with any acute debilitating disease, including experimental malignancy; and (3) that the alterations in coagulability, as studied in tumor bearing rabbits, tend to run a course parallel with the degree of effort expended by the animal in resisting the inroads of the tumor, more particularly in resisting metastases by way of the blood stream.

In the histological examination of organs, particularly the lungs, of rabbits killed during the progress of malignant disease and in the early metastatic period, it is not uncommon to find tumor-cell emboli lying within the lumina of arterioles surrounded by a thrombus. This has been noted by others in cases of human malignancy.[2, 3] It seems possible that the wall of blood clot, that forms about tumor emboli when they first lodge in a capillary bed, may impair the chances of the tumor cells for taking root in their new position, and, moreover, that the degree of firmness and contraction of which the clot is capable is a factor of considerable importance in determining the survival or death of the cells. It appears likely, therefore, that, not only does the efficiency of coagulation in these rabbits run parallel with the course of events in the disease, but that it also possesses some degree of determining influence.

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[2] Schmidt, M. B., Die Verbreitungswege der Karzinome und die Beziehung generalisierter Sarkome zu den leukämischen Neubildungen, Jena, 1903.

[3] Ewing, J., Neoplastic Diseases, Philadelphia, 1919, Saunders.

237 (2469)

A virus, probably of rabbit origin, encountered during intratesticular transmission experiments.

By C. H. ANDREWES and C. PHILIP MILLER, Jr.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

As inoculation into rabbit testicles has been found to be a useful method for the propagation of certain viruses, this procedure was employed in an attempt to transmit a hypothetical virus of rheumatic fever to animals. Whole blood obtained from patients during the acute stage of the disease was injected into the testicles of rabbits. These were removed aseptically after 4 days, ground with sand and Locke's solution, and centrifuged at low speed for 2 minutes; 1 to 2 cc. of the supernatant fluid was injected intratesticularly into other rabbits. In 7 series transfers were carried on in this manner every fifth day for five or more generations. The rabbits used in the first few generations of each series had been previously injected subcutaneously with benzol in order to lower their resistance; later, normal rabbits were employed. Aerobic and anaerobic cultures of the testicles made at each transfer were almost invariably sterile; and dark field examination revealed no spirochetes.

In three series the rabbits showed evidences of an infection which appeared between the third and seventh transfer. This infection was manifested clinically by fever and by swelling and congestion of the testicles. Microscopic examination of sections of these testicles stained with eosin and methylene blue showed profuse interstitial accumulations of endothelial cells, lymphocytes and polymorphs in varying proportions; also marked disturbance of spermatogenesis. A characteristic feature was the presence of pink-staining bodies within the nuclei of large cells in the interstitial spaces. These bodies were indistinguishable from the so-called nuclear inclusion bodies found in the lesions produced by the herpetic and other ultramicroscopic viruses.

What appeared to be a virus could thus be propagated indefinately in either benzolized or normal rabbits. Intradermal inoculations of testicular emulsions produced a slightly elevated erythematous lesion after 5 or 6 days. Intrathoracic inoculations near the heart led to an acute fibrinous pericarditis and a localized myocarditis. Nuclear inclusion bodies were found in the lesions of the skin, pericardium, and myocardium.

The serum of rabbits inoculated 14 days previously was found to neutralize the virus *in vitro* while that of normal rabbits and of patients convalescent from rheumatic fever failed to neutralize it. The virus could be preserved in 50 per cent glycerine for 20 days and for at least 5 days after freezing and drying.

The technique employed was the same as that described by Rivers and Tillett[1] in their studies on Varicella. The apparent identity of the clinical and histo-pathological pictures in our rabbits with that produced by the virus isolated by them suggested the possible identity of the two viruses. With their coöperation, therefore, we conducted a series of cross immunity experiments. It was then found that rabbits inoculated with one virus were subsequently immune to the other, and that sera of rabbits immune to one virus would neutralize the other *in vitro.* We were, therefore, forced to the conclusion that we were dealing with the same virus, which almost certainly bore no relationship to rheumatic fever.

In order to determine whether the virus was of rabbit origin, the same technique was employed with six control series of rabbits in which normal rabbit blood was used as the original inoculum. Since Rivers and Tillett[2] had encountered evidence suggesting that the infection might be contagious amongst rabbits, every possible precaution was taken to prevent contamination of this control stock with the virus previously studied. All infected animals were killed, the cages sterilized, and the premises thoroughly cleaned with disinfectants. Three of the control series received benzene subcutaneously; three did not. What was apparently the same infection appeared in two series, after the third and fourth transfers respectively. One positive series had received benzene, while the other had not. The identity of this virus with that previously encountered has been established by finding similar nuclear inclusions and by cross immunity tests.

It thus seems highly probable that the lesions induced by successive transfers of rabbit testicles originally inoculated with blood from rheumatic fever patients, and from normal rabbits are identical; and that the virus inciting these lesions is of rabbit origin. Its identity with the virus discovered by Rivers and Tillett seems also to be established; these authors on other grounds have already suggested a possible rabbit origin.[1, 2] These facts must be kept in mind in undertaking any future work in which this technique is used, as well as in interpreting past work in which the method of testicular transmission of the so called filtrable viruses has been employed.

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[1] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1923, xxxviii, 673.

[2] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1924, xxxix, 777.

238 (2470)

The prevention and cure of rickets by means of bile.

By ROBERT KAPSINOW and DEBORAH JACKSON.*

[*From the Department of Surgery and the Department of Pediatrics, Yale Medical School, New Haven, Conn.*]

Since it has now become well established that cod liver oil prevents and cures rickets, the question naturally rises whether products of mammalian liver, in particular, bile, might not exert a similar action. The bile used was freshly obtained gall bladder bile from the pig. The animals used in the experiments as test subjects for the effects of bile were rats about 32 days old. The rats were divided into two groups.

In group I bile was added to the diet No. 3143 used by McCollum, Simmonds, Shipley, and Park[1] for the production of rickets in the rat.

The diet with the bile added was then fed for a period of 35 to 36 days with the object of determining whether the bile would prevent the development of rickets. In group II the rickets producing diet without the addition of bile was fed for 35 days, by which time, as is now well known, well marked rickets develops in the rat. At the end of that time bile was added to the diet and the diet plus the bile fed for 15 days. The idea was to determine if bile would exert a curative action on rickets already established. The rats on the diets containing bile were abundantly controlled by rats on the same diet without the addition of bile.

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* Introduced by Edwards A. Park.

[1] McCollum, E. V., Simmonds, Nina, Shipley, P. G., Park, E. A., *J. Biol. Chem.*, 1921, xlvii, 507.

The following results were obtained:

Group I. 7 cc. of pig bile per day prevented the development of rickets. On gross examination of the animals the bones were found normal. On microscopic examination the cartilage proved completely calcified. In the case of some animals, however, the trabeculæ showed here and there traces of osteoid in excess of the normal. 3 cc. of pig bile per day did not completely prevent the development of rickets. 1 cc. exerted a well marked inhibitory action but did not prevent the development of the condition.

Group II. 7 cc. of pig bile per day completely cured rickets when fed for a period of 15 days. 5 cc. and 2.5 cc. of bile per day did not completely cure the rickets. 5 cc. of bile exerted a manifestly greater curative action than 2.5 cc.

The controlled rats all showed rickets.

Further experiments on the effect of bile in the prevention and cure of rickets are now in progress and will be reported later.

239 (2471)

Hyperplasia in the brain of amblystoma.

By H. S. BURR.

[*From the Department of Anatomy, Yale School of Medicine, New Haven, Conn.*]

An experimental study of the effect on the brain of *Amblystoma* of the transplantation of an additional nasal placode has given some rather curious and interesting results. When a right nasal placode from an *Amblystoma* embryo is transplanted into the reentrant angle between the optic cup and nasal placode of a host, there results either complete fusion, partial fusion, or the maintenance of complete integrity of the two placodes. When the union is more or less complete, a majority of the experiments show an augmentation in size of the olfactory nerve through the addition to it of axones derived from the neuro-epithelium of the transplant. The ingrowth of the enlarged olfactory nerve trunk into the cerebral hemispheres results in an increase of the cellular content of the olfactory bulb amounting to approximately 30 per cent. No such increase occurs in those instances where the transplant has made no contribution to the olfactory nerve.

In the experimental embryos where the transplant did not fuse with the placode of the host, the outgrowing fibers from the neuro-epithelium of the transplant pursued either one of two courses. In a majority of cases they established connection with the pars dorsalis thalami with resulting hyperplasia of a restricted region in the central gray. Several experiments resulted in the axones from the transplant joining the ophthalmic branch of the Vth cranial nerve, ascending along it and terminating in the ophthalmic ganglion. Here again hyperplasia of the cellular elements in the ganglion occurred.

It is suggested that the growth of axones into a field of growing neuroblasts is a factor in producing cell proliferation, the degree of which is dependent upon the number of ingrowing nerve units. More than the normal number of these can produce hyperplasia of the region.

240 (2472)

The effect on the involuntary nervous system of arsenicals and the salvarsan group.

By E. F. MUELLER and C. N. MYERS.

[*From the Department of Dermatology and Syphilology, College of Physicians and Surgeons, Columbia University, New York City.*]

I.

Investigations on the effect of nonspecific therapeutic agents by one of us (E. M.) have shown that these all become effective through their action on the involuntary nervous system. These findings were mainly obtained from observations of intradermal injections of Aolan, a nonspecific lact albumin preparation. After these injections were administered intradermally, an acute leucopenia of the peripheric vessels becomes evident for approximately half an hour. Extensive studies on intradermal injections led to the explanation of these phenomena. Every acute decrease of leucocytes in the peripheric vessels corresponds with an increase of leucocytes, more particularly of the neutrophile polynuclear type, in the vessels of the splanchnic area. This acute displacement of leucocytes is a manifestation which shows that there is a stimulus operating upon the parasympathetic portion of the vegetative nervous system. The cause of this stimulus may originate in different organs. The result, especially in the described acute leucopenia—possibly there are also other secondary results, as yet unknown to us—is a vasodilation in the visceral region. It is assumed from earlier experiments that this vasodilation in turn causes an increase in leucocytes in the distended vascular region and a corresponding impoverishment, or leucopenia in the peripheric blood.

II.

Further studies upon a similar acute leucopenia observed immediately after intravenous injections of salvarsan and neosalvarsan in man became possible in connection with the above findings which revealed that such a decrease is always a result of a stimulation of the parasympathetic nervous system.

The first observations along these lines have shown that following every injection of salvarsan in a human being there is a decrease in the leucocyte count; this decrease as a rule does not exceed 1/3 the original count and does not persist longer than 10 minutes. In some patients, presenting reactions immediately after treatment (so-called table reactions) the decrease of leucocytes was greater than generally observed; the decrease in such cases frequently amounting to 7/10 and more. Sometimes unusually low counts (one to two thousand) were observed. The leucocyte count increased again as the reaction gradually disappeared. The results obtained from observation of clinical material will be found in another article.

These changes of the leucocyte curves became more interesting when it was found that after injections of silver salvarsan there was practically no difference in the numbers of white blood cells before and after injection. This observation together with the well-known fact that the so-called nitritoid crisis is very rarely observed after injections of silver salvarsan showed that these manifestations are closely related to the immediate condition of the involuntary nervous system. Only patients presenting angioneurotic symptoms after injections of salvarsan showed an exception to this rule in more or less decreasing leucocyte curves, also after administration of silver salvarsan; thus proving that in these patients there must be a previous disturbance or hypersensitiveness of the involuntary nervous system. It is not known whether this disturbance is caused constitutionally or by the syphilitic infection. The involuntary nervous system causes not only the acute blood changes but also the manifestations known as symptoms of angioneurotic shock.

The remarkable difference in the effect of these two members of the salvarsan group on the leucocyte count led to studies in man which will be published as soon as our series of investigations has been completed. Another series of studies was undertaken concerning the effect of toxic and unusual compounds of the salvarsan group on rats. The first experiments showed the effect of fatal doses of properly alkalinized salvarsan and silver salvarsan. The injection of fatal doses did not produce any fundamental difference in the described curves of the blood count while the usual dose given to rats produces an effect similar to that in man. The results of experiments with salvarsan solutions not employed in human therapy were quite different. The injection of the *monosodium salt of* salvarsan is known to be injurious to man in many instances; the reaction following such injections are often severe and dangerous. In experimenting with these solutions in rats it was found that a large decrease of the leucocyte count took place. Comparison of the observations after injections of this drug with those following injection of the *disodium salt of* salvarsan showed that the decrease of leucocytes in much larger in the case of the *monosodium salt* than in that of the *disodium salt.*

Further studies were undertaken with *concentrated acid salvarsan* solutions. It is known, that concentrated acid salvarsan solutions are usually fatal in humans and have been responsible for the death of many patients. Experiments in rats also demonstrated a considerable fall in the number of leucocytes after giving such acid preparations. These experiments were not always uniform; some of the animals even dying during the injection. In such cases no leucocyte reaction was observed. If death did not result immediately the change in blood cells was striking, their number at times decreasing to below one thousand.

Specimens of blood were taken from the tail vein and from the heart of the living animal under controlled conditions. Normal counts were made in every instance at periods approximately 30 seconds apart. Specimens of blood were obtained from the liver of the animals which died as a result of the shock.

III.

The experiments in rats together with the observations in man following the injection of various salvarsan preparations in connection with the earlier findings on the relation between leucocytes and the involuntary nervous system led to the following conclusions: Every intravenous injection of salvarsan is followed by a slight decrease of leucocytes in the peripheric vessels of the entire body indicating a small reaction of the involuntary nervous system. The decrease is great in patients who develop manifestations of the so-called nitritoid crisis.

Intravenous injections of silver salvarsan are as a rule not accompanied by any change in the leucocyte count, thus indicating that there is no reaction or disturbance of the involuntary nervous system or of the vascular system of the body.

Fatal doses of the salvarsan solutions usually employed do not produce fundamental differences in the reaction of the autonomic nervous system. Monosodium salt and acid solutions of salvarsan always produce a great disturbance of the involuntary nervous system. The parallelism of the leucocyte count and the frequency and character of the clinical reactions following these different preparations shows the relation between the manifestation in the blood and the reaction so far characterized only by the clinical picture. Further it shows that fatal or dangerous disturbances of the autonomic nervous system are not caused by the drug itself but by the use of solutions whose physical characteristics are not properly adjusted to the body fluids, or by previous disturbances of the involuntary nervous system in certain individuals.

241 (2473)

The effect of insulin upon the rate of fermentation of glucose by yeast.

By JANET G. TRAVELL and JEANETTE ALLEN BEHRE.*

[*From the Department of Chemistry, Cornell University Medical College, New York City.*]

The possible effect of insulin upon the rate of fermentation of glucose by yeast was studied. Five samples of insulin were tested in twelve experiments. The insulin used was supplied by Eli Lilly and Co., to whom we are indebted for this courtesy. Merck's highest purity glucose was used. The yeast employed was fresh baker's (Fleischmann's) yeast. The same volume of a solution of glucose was allowed to stand with measured quantities of yeast suspension at about 35° C, with and without the addition of insulin. Portions of the solutions were withdrawn from time to time and determinations made of the amount of sugar remaining as the action of the yeast proceeded. In some of the experiments a phosphate buffer solution was added. A summary of two out of the twelve experiments follows.

Starting with 100 cc. of 1 per cent glucose, and using 10 cc. of a 10 per cent suspension of yeast and 10 units of insulin in 10 cc. of water and no buffer, it was found that the sugar fell in three and a half hours from 0.80 gm. per 100 cc. of the original solution to 0.38 gm., in the solution without insulin, and from 0.81 to 0.37 gm. in the solution with insulin. In another case, starting with 50 cc. of 5 per cent glucose, using 10 cc. of 10 per cent yeast suspension, 40 units of insulin and 10 cc. of a phosphate buffer solution, the sugar fell after nineteen hours from 2.54 to 1.63 gm. per 100 cc. of the original solution, in the flask containing no insulin, and from 2.6 to 1.64 gm. where insulin was present.

These results are representative of seven experiments carried out with four samples of insulin, one of which contained no preservative and the others either toluene or trikresol. In using a fifth sample of insulin, one which contained chloroform as a preservative, a slightly greater loss of sugar was observed in the solution containing insulin than in the one where no insulin was present. This amounted only to a difference of about 10 to 15 per cent of the original glucose after nineteen hours with yeast, and since the result was not duplicated with other samples of insulin we may assume that it was not due to the insulin itself.

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* Introduced by Stanley R. Benedict.

The failure of insulin to exert any influence upon the rate of destruction of glucose by yeast would seem to furnish evidence in favor of the view that the action of insulin is not upon the glucose molecule itself. Were the glucose molecule altered through contact with the insulin it would seem probable that an alteration in the rate of fermentation should be detectable even though the change produced by the insulin were only in the nature of a shift in equilibrium in the α, β and γ glucose molecules present.

We are indebted to Professor S. R. Benedict, at whose suggestion the experiments were undertaken.

242 (2474)

Studies of the physiological behavior of glyceryl tri-margarate (Intarvin). 1. Tests of the effects of Intarvin, in successive generations of albino rats, when added to balanced natural diets.

By HATTIE L. HEFT, MAX KAHN, and WILLIAM J. GIES.

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On August 2, 1923, we began two independent series of feeding experiments on albino rats (in tests continued through successive generations), to determine whether or not Intarvin, added to an ordinary balanced laboratory diet, in quantities ranging from five to twelve per cent of the total daily food intake, would induce any evidences of toxicity.

Eight normal female rats from one litter were originally separated into groups of four. One of two males, of practically the same size and vigor from an unrelated litter, was added to each of these two groups. One group received a balanced natural diet with a definite proportion of Intarvin mixed with the meat ration. The other group received the same natural diet under identical conditions, with rendered lamb fat instead of Intarvin added to it. In each successive generation, thereafter, the maternal rats that received Intarvin were selected from the direct descendants of those that had previously been given the "intarvinated" diet. The control maternal rats in each generation in a series were selected from the direct descendants of those that received a diet containing an addition of lamb fat. The paternal rats in each generation (unrelated to the females) were selected on the same dietary basis as that for the maternal, or from litters on the ordinary diet without addition of fatty matter.

Rats have been carried, in this parallel way, into the fourth generation in one series and into the third generation in another. (The experiments are in progress; and litters in the fifth and fourth generations, respectively, will soon be available.) There has been no discernible effect on the animals thus treated, or on their fecundity. The animals in the third and fourth generations appear to be as completely normal as those with which the tests were started last August.

The tissues of many of the rats have been used in studies of the absorption and distribution of glyceryl tri-margarate. These results, with those of various extensions of the study, will be reported later.

243 (2475)

The effect of light doses of alcohol upon the estrus cycle, and on the number of corpora lutea and prenatal mortality in the mouse.

By E. C. MacDOWELL.

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Stockard and Papanicolaou, Arlitt, Stockard, Hanson, MacDowell[1] have found that alcohol may cause partial or complete sterility in laboratory mammals.

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[1] Stockard, C. R., and Papanicolaou, G. N., *J. Exp. Zoöl.*, 1918, xxvi, 119; Arlitt, A. H., *Psychol. Monog.*, 1919, xxvi, —; Stockard, C. R., *Proc. Amer. Phil. Soc.*, 1923, lxii, 311; Hanson, F. B., and Handy, V., *Amer. Nat.*, 1923, lvii, 532; MacDowell, E. C., *Genetics*, 1922, vii, 117, 427, *J. Exp. Zoöl.*, 1923, xxxvii, 417.

This has been explained both as a result of the elimination of less vigorous germ cells or zygotes, and as an immediate effect of the alcohol upon the activity of the ovary. The present experiments offer a method for analyzing more fully than before the effects of alcohol upon the reproductive processes of a mammal. The following criteria are used here for the first time in a study of this type: (1) the length of the estrus cycle, (2) the number of corpora lutea for given pregnancies, and (3) the proportion of these represented at the subsequent births by living young.

The mice used came from lines that had been inbred for three and four generations by brother to sister mating. Tests and controls from the same litter were kept in the same pen to equalize environmental conditions as far as possible. The alcohol was given by inhalation in pint milk bottles. Before treatment each mouse from one pen was placed in its own bottle; into each test bottle was then inserted a strip of absorbent paper, approximately 37 by 15 cm., wet with 3 cc. commercial (95 per cent) alcohol. The bottle was capped and inverted at once. Into each control bottle was inserted a similar piece of paper without the alcohol; these bottles also were closed and inverted. Each mouse remained in its bottle for 45 minutes. This treatment, starting at weaning (4 to 5 weeks), was given daily. The controls seemed to be unaffected by confinement in the closed bottles. For the first week the alcohol treatment left most mice flat on their sides; later, when most mice could turn over and run off, their behavior still showed an unquestionable effect of the alcohol. A few individuals were left limp, on their sides every time. This treatment is called light in contradistinction to the treatment being used in a subsequent experiment, by which every animal is made dead drunk every time.

Estrus cycle. From weaning till the mice were mated, daily observations were recorded upon the condition of the vagina. After the formation of the vaginal orifice, these observations were based upon the microscopical examination of vaginal smears. The beginning of estrus proper, indicated by the sudden appearance of large cornified epithelial cells (Allen[2]) was chosen as the point by which to measure the length of the cycles. From 41 to 87 smears were taken from each animal; the aver-

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[3] Long, J. A., and Evans, H. M., *Mem. Univ. Cal.*, 1922, vi.

age number per mouse was 66.7. The variations in the number of smears taken depends partly upon the variations in the time of the opening of the vagina and partly upon the time of mating.

The age at the opening of the vagina ranged from 35 to 71 days for the tests, with an average of 54.0 days; for the controls, from 37 to 72 days, with an average of 52.2 days. For the rat, Long and Evans[3] give the average age for the opening of the vaginal orifice as 72 days, with a range from 34 to 109. These data may be considered to indicate that the administration of alcohol for an average of three weeks before the formation of the opening has had no effect upon this process.

Long and Evans report that the first estrus in the rat does not occur before the establishment of the vaginal orifice; that the two processes may be synchronous, or the first estrus follow the opening. Of the thirty-nine mice under consideration, only one, a test animal, showed signs of estrus at the time of the opening of the vagina. The age of the tests at the first estrus ranged from 47 to 107 days, with the average of 71.9 days; the age of the controls ranged from 51 to 115 days with the average of 68.2 days. This difference is probably due to random sampling as it is only about twice its probable error (6.6 ± 3.4).

The closest control of living conditions is obtained when the data on the length of the estrus cycle are arranged by pens. The closest control upon genetic differences is obtained when the data are arranged according to strains, for each strain includes closely related litters. Table I gives the data arranged in both the above ways.

This table shows that in four of the six pens, the average length of cycles was somewhat more for the tests; that in three of the four strains the average length of the cycles was less for the tests. The total shows practical identity. As far as these data are concerned, it is apparent that the treatment given did not increase the length of the estrous cycle.

Corpora lutea of pregnancy and prenatal mortality. A surgical technique for counting the corpora lutea of pregnancy has previously been described.[4] The count is made during the last week of pregnancy when the corpora lutea corresponding to the ova that gave rise to the pregnancy are the only large and con-

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[3] Long, J. A., and Evans, H. M., *Mem. Univ. Cal.*, 1922, ix.

[4] MacDowell, E. C., *Anat. Rec.*, 1924, xxvii, 59.

TABLE I.
Average length of estrus cycle in treated and control mice.

Treated.					Controls.			Differences.
	Pen No.	No. of mice	No. of cycles	Av. days in cycles	No. of mice	No. of cycles	Av. days in cycles	+ = test cycles longer
Data arranged by pens	439	4	24	7.9	3	25	7.4	+0.5
	441	3	14	10.2	3	22	8.7	+1.5
	437	4	31	8.3	2	13	8.8	—0.5
	449	3	7	12.2	3	12	11.4	+0.8
	445	2	8	11.6	1	5	9.2	+2.4
	443	6	22	9.8	5	12	14.2	—4.4
Data arranged by strains	Strain C57	9	44	9.1	7	40	9.3	—0.2
	42-30	6	21	10.9	6	34	9.6	+1.3
	86	2	21	7.9	1	6	9.5	—1.6
	101	5	20	9.3	3	8	9.8	—0.5
Totals		22	106	9.3	17	89	9.5	—0.2

spicuous structures in the ovaries. Repetitions of this operation do not interfere with the normal activity of the ovaries. In addition to the evidence previously given upon this point, one case may be recorded in which, as a special test, the operation was repeated at ten successive pregnancies. The tenth litter was born 212 days after the first one, an average of 23.5 days apart. In all 77 young were born. Comparing litter size at the beginning and end of the series, the first three litters averaged 7.33 mice; the last three litters averaged 7.33 mice.

The test and control females in a litter were mated to the same normal male. The number of alcohol treatments given before the first conception averaged 118.8 per mouse, with a range from 76 to 205. No treatment was given between an operation and the birth of the litter. The young were killed on the day of birth and the female returned to her mating box. The daily treatments were resumed on the following day. The results are based upon 107 counts of corpora lutea of pregnancy by operation and 8 counts by autopsy. For the treated mice the average number of corpora lutea per pregnancy was 9.95; for the controls, 9.90.

By subtracting the number of young found at birth from the number of the corresponding corpora lutea a fair measure of the amount of prenatal mortality is obtained. This includes ova that were not fertilized, as well as zygotes that died at any stage before birth. In the previous study it was shown that the operation itself does not play a part in this prenatal mortality. In most cases mice found dead at birth appear to have been alive up to the point of birth but failed to survive the process, although there is no certain exclusion of the possibility that some of those found dead at birth had recently died *in utero*.

The average number of young that were expected at birth and not found, dead or alive, was 3.48 per litter for the tests and 3.44 for the controls. The average number of expected young that were not found alive was 3.82 per litter for the tests and 3.97 for the controls. The number of young found alive gave the average of 5.79 per litter for the tests and 5.79 per litter for the controls.

TABLE II.

Summary of data on the effect of light doses of alcohol upon the number of the corpora lutea of pregnancy and the amount of natal and prenatal mortality.

Strain	C57		42-30		86		101		Total	
	Test	Contr.	Test	Contr.	Test	Contr.	Test	Contr.	Test	Contr.
No. of mice	9	7	6	5	2	1	4	1	21	14
No. of counts										
a, by operation	28	23	16	15	8	9	6	2	58	49
b, by autopsy	4	1	2	0	1	0	0	0	7	1
Av. No. corpora per pregnancy	9.87	10.12	9.16	8.40	11.66	12.00	10.16	9.00	9.95	9.90
No. of litters	28	23	16	15	8	9	6	2	58	49
Av. No. young born alive per litter	5.85	5.43	5.37	5.13	6.12	7.88	6.17	5.50	5.79	5.79
Prenatal mortality per litter	3.28	3.87	3.18	2.60	4.37	3.88	4.00	3.00	3.48	3.44
Natal and prenatal mortality	3.67	4.34	3.75	3.33	4.37	4.22	4.00	3.50	3.82	3.97

Table II presents the above figures with other relevant data, including subdivisions according to strains. The evidence all leads to the conclusion that the alcohol has had no effect upon the number of corpora lutea formed or upon the survival of the zygotes.

Conclusion. It is concluded that the alcohol treatment has had no effect upon the reproduction of the mice studied when judged by the following criteria: (1) age of opening of the vaginal orifice; (2) age at first estrus; (3) length of estrus cycle; (4) number of corpora lutea per ovulation; (5) prenatal and natal mortality; (6) number of living young per litter.

244 (2476)

The food value of intarvin.

By E. M. BENEDICT, W. S. LADD, M. L. STRAUSS, and
RANDOLPH WEST.

[*From the Department of Medicine, Presbyterian Hospital,
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In order to ascertain whether the synthetic fat, intarvin, composed of 17 carbon atom fatty acids, could be used by the body as a food, observations were made on two diabetic patients and four normal individuals. This was done in conjunction with work on dogs reported in another communication.[1] It is the purpose of this paper to present the data obtained from feeding experiments on two of the normal individuals. The other two normals were unable to eat enough intarvin to yield results sufficiently conclusive for interpretation.

Discussion.

The diabetic patients were upon restricted diets for therapeutic reasons. These diets were insufficient in calories to maintain nitrogen equilibrium and weight. They were receiving insulin. Additions of 80 and 100 grams of intarvin showed no increase in ketone bodies and was followed by a lowering in nitrogen excretion. The period during which intarvin was given was for one patient five days, for the other ten days. The results are not decisive enough to make it worth while presenting the protocols. Difficulty was experienced in getting the patients to take the intarvin and it had to be discontinued.

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[1] Benedict, E. M., and West, R., Intarvin in phlorhizinized dogs, Proc. Soc. Exp. Biol. and Med., 1924, xxi, 225.

E. M. B. *	Diet	Urine Nitrogen	Ammonia terms 0.1 N HCl	Total Acetone Bodies	Organic acids (corrected) terms 0.1 N HCl	Volume	pH	Body Weight	R. Q.
Feb.		gm.	cc.	gm.	cc.	cc.		kg.	
5 6	100-60-150	9.00	277	0.127	443	930	6.2	62.2	
6 7	25-60-179	11.25	437	0.488		1500	5.9	60.5	0.76
7 8	25-60-179	12.68	558	2.060	728	1160	5.4	61.4	
8 9	25-60-179	12.95	712	4.040	1024	1130	5.5	60.7	
9 10	25-60-179	12.17	698	5.500	1187	1510	5.6	60.0	0.69
10 11	+Intarvin 125 25-60-54	11.48	840	1.510	821	1400	5.7	60.6	
11 12	Intarvin 25-60-54	9.14	461	1.200	726	1465	5.8	60.3	0.71
12 13	No Intarvin 25-60-54	10.46	671	1.630	701	900	5.8		

*On Feb. 4-5 specimen lost.

M. L S.

4 5	100-60-150	10.08	165	0.155	498	480	5.5	67.7	
5 6	100-60-150	specimen lost				1220		67.7	0.80
6 7	25-60-179	14.64	398	0.866	675	770	5.4	66.8	
7 8	25-60-179	15.61	166	5.040	1166	980	5.2	66.2	
8 9	25-60-179	14.62	754	7.080	1442	1060	5.1	65.8	
9 10	25-60-179	17.15	830	4.660	1131	900	5.2	65.5	0.73
10 11	Intarvin 125 25-60-54	12.15	986	2.740	913	795	5.3	65.5	
11 12	Intarvin 25-60-54	9.97	930	2.080	816	1050	5.3	65.5	0.70
12 13	No Intarvin 25-60-54	12.50	900	3.430	984	735	5.4		

The normal subjects were placed upon a diet the total caloric value of which was designed to maintain nitrogen equilibrium. After a preliminary period of two days the carbohydrate and fat were shifted isodynamically to produce ketosis. This occurred together with an increase in nitrogen excretion. Upon the fourth day of this high fat-low carbohydrate diet intarvin was substituted for the bulk of the food fat.

From the data of these tables we may conclude that the use of intarvin resulted in:

1. Protein sparing action.
2. Decrease in ketosis.
3. A lowering of the respiratory quotient suggesting that a fat was being burned.

Thus intarvin was used by the body as a food and did not produce ketosis.

There is, however, no proof that intarvin reduces ketosis *per se,* that is, by promoting the complete oxidation of ketone bodies or body fat as does carbohydrate.

Examination of the stools in all cases showed that 92 to 98 per cent of food fat and of intarvin was absorbed.

The experiences of these individuals in taking intarvin should not pass without remark as to its gustatory and esthetic qualities as a food. All individuals found it decidedly unpalatable. Its peculiar odor and physical properties made it distressing for all to take. Some had less difficulty. A great many methods of taking it and combinations with other foods were tried, none of which were satisfactory. Anchovy paste and tomato catsup were the most effective in hiding the taste. The most agreeable methods of taking it were as follows: to eat it dry and wash down with black coffee; to mix with scrambled eggs or with roquefort cheese salad.

In large amounts it caused gastric distress and disagreeable eructations of gas for several hours. This proved to be the case whether taken alone or with condiments.

245 (2477)

Vomiting reflex from stomach after vagotomy.

By A. O. SHAKLEE and F. O. ANDERSON.

[*From the Department of Pharmacology of the St. Louis University, St. Louis, Mo.*]

In 1889 Openchowski[1] published the conclusion that the afferent paths from the stomach to the vomiting center are confined to the vagi; and the same belief has since been repeatedly expressed by eminent pharmacologists, at least up to 1922. Last summer, in trying to have a class of students demonstrate this, we were surprised to find that all the vagotomized dogs to which zinc sulfate solution was administered, vomited rather promptly, and in some cases, before the controls. We have repeated the experiments with the same results; all the vagotomized dogs tried vomited promptly, some immediately.

246 (2478)

Drainage in intestinal obstruction.

By H. B. EISBERG.

[*From the Department of Experimental Surgery, University and Bellevue Hospital Medical College, New York City.*]

The following experiments were carried out to establish the value of intestinal drainage and other procedures for intestinal obstruction.

These obstructions were termed early, intermediate, or late in a previous communication. Classifications were arbitrarily based upon the duration and severity of the associated toxemias.[1]

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[1] Openchowski, T., *Centblt. Physiol.*, 1889, iii, 1

[1] Eisberg, H. B., *Ann. Surg.*, 1921, lxxiv, 584.

Experimental technic	Total animals used	Duration of obstruction before corrective procedure	Corrective operative procedure	Average duration of life
Duodenal fistula	4	No obstruction	No operative procedure performed.	70 hours*
Jejunal fistula	2	No obstruction	No operative procedure performed.	1 week*
Duodenal obstruction				
a. Severed gut type	2	24 hours	No drainage; reconstruction after 23½ hours.	4 weeks†
	3	24-72 hours	Duodenal drainage after 72 hours.	46 hours after drainage
	2	72 hours	Duodenal drainage after 96 hours.	6 hours after drainage
b. band type	3	24-72 hours	Removal of band after 24 hours; no drainage.	4 weeks†
Exclusion and occlusion of segment				
a. in duodenum	2	1-5 hours	Removal of segment after 5 hours; immediate reconstruction of canal.	4 weeks†
	3	1-10 hours	Removal of segment after 7 hours; drainage of duodenum; reconstruction of canal after 24 hours.	20 hours after reconstruction
b. in ileum	2	1-12 hours	Removal of segment after 6 hours;	
	2	18-24 hours	a. immediate drainage.	4 weeks†
			b. Drainage after 24 hours.‡	4 weeks†
c. in ileum (Segment placed between fascial planes of abdomen)	3	18-24 hours	Removal of segment after 18½ hours; immediate drainage.	4 hours after drainage
d. in ileum (Segment left outside abdomen)	3	72 hours	Removal of segment after 20 hours; immediate drainage.	10 hours after drainage
	2		Removal of segment after 3 days; reconstruction of canal.	4 weeks†
e. in colon	3	36 hours	Removal of segment after 10 hours; immediate drainage.	4 weeks†

*Duration of life is equal to that of animals that have been obstructed at these same points and have not developed toxemia.

†These animals were observed to be in good condition at end of 4 weeks.

‡Animals remained toxic for 24 hours until relieved by ileostomies.

247 (2479)

Researches on the antigenic modifications of bacillus typhosus.

By D. COMBIESCO* (Bucharest).

[*From the Department of Pathology, Johns Hopkins University, Baltimore, Md.*]

Our investigations[1] concerning the vaccination of animals through buccal and cutaneous routes have led us naturally to the study of the antigenic modifications of certain micro-organisms, especially of *B. typhosus* and *B. paratyphosus B* in contact with the body fluids. We shall give on this occasion some of our results.

As early as 1891, Metchnikoff[2] had seen that the vibrio Metchnikovi, grown in the exudate of the immunized individual could form either clumps or give a culture made up of separate organisms, according to the previous environment of the microbe. Later Metchnikoff and Bordet[3] reported that "a vibrion chloreæ (vibrion of eastern Prussia of highly accentuated virulence) was only partially affected by the serum of a horse immunized against cholera, after having been exposed for a certain time to the action of phagocytic protoplasm" "*protoplasma phagocytaire*". Grassburger and Shattenfroh,[4] in their work on symptomatic anthrax, found that the serum obtained by the injection of virulent exudate agglutinated the microbe growing in this exudate, but not the microbe grown upon artificial medium. Bordet and Sleeswijk,[5] in their work with *B. pertussis,* showed that by changing the nutritive medium, one can create, using parts of the same culture, races distinguished serodiagnostically by their agglutination reactions, incapable of uniting with the same antibodies, and whose injection into animals gives different immune-sera. Gay and Claypole[6] found the same thing for *B. typhosus.* Their cultures were grown either upon agar-agar or 10 per cent blood agar.

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* Introduced by W. L. Holman.

[1] Combiesco, D., *Compt. rend. Soc. de biol.*, 1923, 88, 904; the same, 1923, 89, 634, 637, 640.

[2] Metchnikoff, E., *Ann. Inst. Pasteur,* 1891,, (quoted by Bordet).

[3] Metchnikoff and Bordet, *Ann. Inst. Pasteur,* 1896, x, 1931.

[4] Grassburger and Shattenfroh, *Sitzungsberichten der Kaiserlichen Akademie der Wissenschaft in Wien,* mathem. Naturw. Klasse, 1905.

[5] Bordet and Sleeswijk, *Ann. Inst. Pasteur,* 1910, xxiv, 776.

[6] Gay, F. P., and Claypole, E., *Arch. Int. Med.,* 1913, xii, 621.

We used, throughout these experiments, the same strain of *B. typhosus;* one that we obtained by the courtesy of Dr. Holman of the Department of Pathology. The cultures were grown on ordinary agar slants for 24 hours at 37° C. and emulsified in physiological salt solution, 10 cc. for each slant. The emulsions were mixed so as to have as nearly as possible the same quantity of organisms in the same volume. One part of this emulsion was used for the inoculation of a series of rabbits. Another part was mixed with oxalated blood of normal rabbits (or the blood of rabbits partially treated as discussed below), and kept for 5 hours at 37° C., then inoculated into another series of rabbits, taking care that the number of microbes injected into each rabbit was equal to that inoculated into the rabbits of the first series. Space does not permit us to give all the details, we will content ourselves for the time being with giving the more conclusive results, shown by the examination of some of the animals used.

Rabbit No. 5 (1850 gm.) received a first intraperitoneal injection of 1 cc. of the emulsion heated one hour at 60°, on the third day the same dose, intravenously, on the fifth day the same dose of living bacteria, also intravenously; this dose being repeated on the 7th and 9th days.

Rabbit No. 3 (2025 gm.) on the same days received the same inoculations as rabbit No. 5; this time, the emulsion of bacteria was exposed to the action of 1 cc. oxalated blood. The first time the blood was that of a normal rabbit; at the second injection the blood from rabbit No. 3 which had received the above mixture two days before was taken—on the third, fourth and fifth inoculations the blood was always taken from rabbit No. 3 or another that had received the same treatment.

Five days after the last inoculation, the serum of rabbit No. 5 agglutinated very well the *B. typhosus* grown on ordinary agar in a dilution of 1:200 (examined after 2 hours at 37° C.). Under the same conditions the serum of rabbit No. 3 did not agglutinate the *B. typhosus* at all.

Ten days after the last injection the animals were bled again; the examination made under the same conditions showed: for rabbit No. 5, a clear agglutination titre of 1:10,000, with limit of 1:40,000. For rabbit No. 3, absence of agglutination at dilution of 1:20, and a slight agglutination at 1:100 (giving the same appearance as in rabbit No. 5 at 1:40,000). We repeated the agglutination experiments but this time we made an emulsion of agar cultures, not in salt solution as above, but in citrated plasma of the normal rabbit, or the plasma defibrinated by shaking. The emulsion was kept at 37° C. for 3 hours before being added to serum dilutions. The results: the serum of rabbit No. 3, which does not agglutinate the bacteria cultivated on ordinary agar and emulsified in physiological salt solution, agglutinates very well up to 1:10,000, the same microbe when it has been affected by plasma. We worked with this plasma in dilution 1:5 and 1:10, and observed differences with the different solutions as we will discuss.

It is quite easy to find normal plasma which does not give any agglutination and such plasma naturally must be used in such studies.

Another group of animals received only two inoculations under the skin at 7 day intervals. Some of the animals "a" 0.5 and 1.0 of the emulsion of living bacteria; the others "b" the same amount of emulsion after exposure to normal oxalated rabbit blood for 5 hours at 37°. Ten days after the last inoculation the serum of rabbits "a" agglutinated very well (1:200 to 1:400) the bacteria grown on agar and emulsified in physiological salt solution, the serum of rabbits "b" either did not agglutinate at all, or at a dilution of 1:20, a very slight agglutination that one need not consider. The same experiment made with the emulsion in oxalated plasma or defibrinated plasma of normal rabbits in the dilutions mentioned above gave the following results:

1. The serum of rabbits "a" agglutinates the microbes affected by the plasma but with an intensity varying according to the whole plasma, or different dilutions of the plasma or one or the other plasma. For instance: the serum of certain rabbits agglutinated better the microbes emulsified in the oxalated plasma, diluted to 1:10, than the microbe in the same undiluted plasma. One agglutinates very well the bacterial emulsion in the oxalated plasma, and not at all, or very poorly, the bacteria emulsified in the defibrinated plasma.

2. The serum of rabbits "b" under the same conditions, agglutinates very well the organisms that are exposed to the influence of the plasma, showing, however, a certain variation according to the plasma and the dilution of plasma used. The

serum of normal rabbits, and physiological salt solution did not agglutinate the modified bacteria at all.

So under certain conditions, antigenic modifications of typhosus are evident.

248 (2480)

Researches on the antigenic modifications of bacillus paratyphosus B.

By D. COMBIESCO* (Bucharest).

[*From the Department of Pathology, Johns Hopkins University, Baltimore, Md.*]

In this series of experiments we have sought to determine whether *B. paratyphosus B* undergoes the same antigenic modifications as *B. typhosus*[1] when it is influenced by the fluid of the organism; in this particular case in contact with the oxalated blood of the normal rabbit.

In these experiments, emulsions of 24 hour agar cultures were heated at 60° C. for one hour. Six rabbits, "a", received two subcutaneous injections of this vaccine 7 days apart (0.5 and 1.0 cc.). Four rabbits, "b", were inoculated, time and quantity the same, with emulsion that had been kept in contact for 5 hours at 37° C. with oxalated blood of normal rabbits. Five days after the last injection the four rabbits of series "b" and two of series "a" were bled. The serum of rabbits "a" agglutinated the original *B. paratyphosus B* after 24 hours at 37° C. in dilutions of 1 :100 to 1 :200. That of rabbits "b", under the same conditions, either did not agglutinate at all, or in a dilution of 1 :20, which has no significance. Ten days after the last inoculation the animals were again bled, and we tested their agglutinins as before. The serum of rabbits "a" agglutinated in a dilution of 1 :200 to 1 :400, while that of rabbits "b" agglutinated in dilution 1 :20, or not at all.

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* Introduced by W. L. Holman.

[1] Combiesco, D., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 490.

At the same time we tested the precipitins, using two antigens, one being the filtrate of ten day bouillon cultures of *B. paratyphosus B;* the other being prepared from emulsions of 24 hour cultures on ordinary agar shaken for 24 hours, and filtered like the first antigen, using a mandler filter candle, Nos. 10 and 11. Neither the serum of rabbits "a" or "b" precipitated in the presence of these antigens.

With the above two antigens, and a third prepared by heating a bacterial emulsion for one hour at 60° C. we tested for specific sensitizers by the Bordet-Gengou method. We made dilutions of the serum up to 1:200, the quantity of the antigen being fixed, and representing half the dose which alone does not fix the complement. The results were observed after one hour at 37° C. and after 24 hours at 6° C. to 7° C. The serum of rabbits "a" sometimes fixed the complement in dilutions as high as 1:100, and almost always in a dilution of 1:50, that of rabbits "b", when used undiluted 0.1 or 0.15 cc. or 0.2 cc. sometimes fixed the complement, but there were rabbits (2 of our 4) whose serum did not fix the complement even when used in this quantity in the presence, of course, of the specific antigen.

In addition we also tested the specific sensitizers by the Bordet-Gengou reaction with the serum of 26 rabbits, of whom eleven (1) were vaccinated with emulsions of T. Bacillus in physiological salt solution, the rest, fifteen, (2) with emulsions treated with oxalated rabbit plasma as we described in the previous paper. The serum of rabbits (1) fix complement in the presence of the specific antigen in a dilution of 1:30 to 1:200, the serum of two rabbits of the eleven, even in a quantity of 0.2 cc. of undiluted serum did not fix the complement at all. These two rabbits had received two subcutaneous injections of 0.1 and 0.2 cc. of emulsion at seven day intervals. The serum of rabbits (2) either fixed the complement when used in quantity 0.10, 0.15, or 0.20 cc. of undiluted serum or not at all, as shown in 12 of the 15 rabbits vaccinated in this way.

However, in the conditions under which we worked, the antigenic properties of *B. typhosus* and *B. paratyphosus B* were modified, although perhaps to a less marked degree, even in their power to influence the organism in the production of fixing sensitizers.

As we have said in a previous article,[2] the resistance of animals tested with a definite lethal dose is at least comparable.

We believe that these experiments can explain up to a certain point why micro-organisms recently isolated from the body, especially from the blood of the patient, are not always agglutinated by their specific serums, and that passage through ordinary culture media is necessary in order to demonstrate their agglutinative properties.

These experiments agree very well with the observations of Patrick[3] concerning the agglutination of *B. typhosus* isolated from the body of patients.

We cannot here speak of microbic mutation in the sense of H. de Vries, these antigenic modifications taking place with microbes heated to 60° C. as well as with living microbes.

This work was done with funds from the Rockefeller Foundation, which we gratefully acknowledge; we also wish to thank Dr. W. G. MacCallum, Dr. W. L. Holman, and Dr. J. H. Brown for the interest they have continually shown in our experiments.

249 (2481)

The rôle of the skeletal musculature in the maintenance of the asphyxial rise of blood pressure during bulbar anemia.*

By HELEN C. COOMBS.

[*From the Department of Physiology, New York University and Bellevue Hospital Medical College, New York City.*]

In an analysis of the cardio-vascular mechanism of the cat by means of the changes in blood pressure and pulse rate taking place under the conditions of medullary anemia caused by temporary ligation of the carotid, vertebral and subclavian arteries, it was considered desirable to ascertain, if possible, the extent of the rôle played by the skeletal musculature in the maintenance of the high pressures which the animals show.

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[2] Combiesco, D., *Compt. rend. Soc. de biol.*, 1924, xc, 752.

[3] Patrick, A., *J. Hyg.*, 1914, xiv, 163.

* The expenses of this research were defrayed, in part, by the Sarah Berliner Research Fellowship.

After a control tracing had been taken, in which the anemic rise of blood pressure was obtained, curare was administered intravenously in doses of 1 mg. per 5 cc. of Ringer's solution. This dose was repeated, if necessary, every five minutes until failure of the respiratory mechanism, after which time artificial respiration was maintained. Intravenous injection of curare is followed by a fall in blood pressure, due to peripheral vasomotor depression. This action is apparently on the ganglion cells (Langley), but Sollmann[1] states that this effect on the circulation passes off within fifteen minutes, while the skeletal muscular paralysis persists. Accordingly, further occlusion was delayed for about fifteen minutes in order that the effects of muscular paralysis alone might be studied. Occlusions of the head arteries were then made as before. While the height of the anemic rise was fair—in some cases double that of the control pressure—the duration before the final fall to spinal level and the number of occlusions obtainable were markedly less than in the normal animal. This is shown in the following table:

Normal				Curare		
June 15, 1923.				March 25, 1924.		
Occlusion number	Length of occlusion	Max. height of pressure	Control pressure	Length of occlusion	Max. height of pressure	Control pressure
	secs.	mm. Hg.	mm.		Control	
1	120	240	100	secs.	mm. Hg.	mm. Hg.
2	180	205	80	200	200	100
3	130	170	80		After curare	
4	160	185	80	90	130	60
5	160	200	75	85	120	65
6	165	230	75	100	120	50
7	170	190	75	95	110	45
8	110	190	70	50	60	30
Six more occlusions were obtained.				No further rise obtainable.		

Another striking feature of the reaction in the curarized animal is the increased latent period after occlusion of the head arteries, before the onset of the anemic rise. Normally, this occurs within one second after occlusion; in the curarized animal, the anemic rise does not begin until ten seconds or more have elapsed. For example, in the experiment of April 21, 1924, the tracing shows that for the control occlusion, one second elapsed between the time the head arteries were clamped off and the onset of the anemic rise, while in the second occlusion, after curare had been previously injected, this same period occupied 11 seconds; in the third occlusion, 11 seconds; and in the fourth and last occlusion, 15 seconds.

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[1] Sollmann, A Manual of Pharmacology, 1922, 383.

Pike[2] has shown that intravenous injection of curare after complete elimination of the medulla brings about a constant fall in blood pressure which is, in general, about equal to the fall observed after division of the dorsal roots of the spinal nerves. Langley[3] confirmed this observation in 1919. This fall is not to be confused with the effects immediately following injection of curare, as its duration persists as long as paralysis of the skeletal musculature. Tillie[4] has stated that the dose of curarin by which the vasomotor nerves are affected in 100 to 300 times as great as that which induces paralysis of the skeletal muscles.

Since it would appear, then, that under the conditions of these experiments, the skeletal musculature and not the vaso-motor mechanism is directly affected by curare, we may be justified in concluding that the skeletal musculature is responsible in some degree for the rapid initiation of the height of the anemic rise, and in a considerable degree for

1. The length of the latent period before response to occlusion.
2. The duration of the anemic rise of blood pressure.

250 (2482)

The influence of suprarenalectomy in rabbits on antitoxin (diphtheria) formation.

By DAVID MARINE.

[*From the Laboratory Division, Montefiore Hospital, New York City.*]

Previous reports by Také and Marine on hemolysin formation in suprarenalectomized rabbits and by Jaffe and Marine on agglutinin (typhoid) formation in suprarenalectomized rats have shown that both hemolysin and agglutinin formation are increased in suprarenalectomized animals. The antibody titer may

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[2] Pike, *Quart. J. Exp. Physiol.*, 1913, vii, 1.

[3] Langley, *J. Physiol.*, 1919, liii, 120.

[4] Tillie, *Arch. exp. Path. u. Pharmacol.*, 1890, xxvii, 1.

Rabbit No.	Sex	Age in days	Date second suprarenalectomy	Dates and amount T.A. injected cc.						Dates and result of Schick tests				Total T.A. injected cc. before neg. Schick	No. days to neg. Schick
				Nov. 12	Nov. 15	Nov. 22	Nov. 30	Dec. 5	Dec. 8	Nov. 19 1/40 M.L.D	Nov. 26 1/80 M.L.D	Dec. 3 1/40 M.L.D	Dec. 10 1/40 M.L.D		
374	M	150	Nov. 5	1/4	1/4	1/4	1/2	1/2		+	+—	—	—	1 1/4	21
463	M	318	"	1/4	1/4	1/4	1/2	1/2		+	+—	—	—	1 1/4	21
458	F	Adult	"	1/4	1/4	1/4	1/2	1/2		+—	+—	—	—	1 1/4	21
454	M	335	"	2/5	1/2	1/2	1	1		+++	++	—	—	2 2/5	21
459	M	Adult	"	1/2	1/2	1/2	1	1	1	+++	++	+	—	4 1/2	28
382	M	621	control	1/2	1/2	1/2	1	1	1	++	++	+—	—	4 1/2	28
487	F	331	"	1/2	1/2	1/4	1/2	1/2	1/2	+++	+	+—	+	2 3/4	pos.
488	F	331	"	1/4	1/4	1/4	1/2	1/2	1/2	++	+++	+++	—	2 1/4	28
490	M	256	"	1/2	1/2	1/2	1	1	1	+++	++	++	—	4 1/2	28
491	M	254	"	1/2	1/2	1/2	1	1	1	+++	+	+—	—	4 1/2	28
493	F	247	"		1/4	1/4	1/2	1/2	1/2	+++	++	+++	+++	2	pos.
492	M	223	"		1/4	1/4	1/2	1/2		+++	+++	—	—	1	18

be two or three times greater than in controls, using the same doses of antigen.

It seemed desirable to test antitoxin formation also in suprarenalectomized animals. The method used consisted of injecting subcutaneously in the right flank the standard toxin-antitoxin mixture (1 cc. equals 0.1 L.+ dose) of the New York Board of Health furnished us through the courtesy of Prof. William H. Park. The Schick tests were made at weekly intervals, using in order the right lower, right upper, left lower and left upper abdomen as sites. The details and results are given in the preceding table.

Of the 5 doubly suprarenalectomized rabbits which survived in good condition, 4 had negative Schick on the 21st day and one on the 28th day. Of the 7 controls, 4 were negative on the 28th day, 2 were still positive on the 28th day and one (492) was negative on the 18th day. It is noteworthy that this control showed a negative Schick earlier than any of the other rabbits and with less toxin-antitoxin. Nothing unusual in the clinical appearance of the rabbit was detected. It died about four months later while attempting ether anesthesia, and at necropsy acute dilatation of the heart with pulmonary edema and congestion were the prominent findings. With this exception it may be stated that the suprarenalectomized rabbits formed antitoxin as rapidly as, and in this series more rapidly than, the controls.

251 (2483)

The follicular hormone of the hen ovary.

By EDGAR ALLEN, J. WILBUR WHITSETT, JOHN W. HARDY and FRED L. KNEIBERT.

[*From the Department of Anatomy, University of Missouri, Columbia, Mo.*]

The demonstration that the follicular hormone from ovaries of swine would satisfactorily substitute for the hormonal function of the ovaries of non-pregnant rats and mice[1, 2] has led us to attempt its extraction from other sources. So far we have obtained it from large, normal follicles of ovaries of swine, cattle, and sheep and from cystic follicles of swine and man, and have injected it into mice, rats, guinea pigs, rabbits, monkeys and human patients. These experiments seem sufficient to establish the expectation that this active substance is not species specific at least among mammals.

After trials in several laboratory mammals we have come to the conclusion that the rat, with both ovaries removed, is the best test animal. It has the following advantages: (1) the time required for the growth reaction of the tissues of the genital tract to the growth stimulus of the injected hormone is short, 36 to 48 hours, thus returning a decisive test after a few injections of a small amount of extract; and (2) the course of the experiment can be followed accurately by microscopic examination of the cell content of the vaginal smear, thus making it possible to use a test animal repeatedly.

As shown in our earlier work, the hormone is present in liquor folliculi from which all cells have been removed. The conclusion drawn was that it is secreted by the follicle cells under the influence of the ovum as the dynamic center.[2] Theoretically, it should be produced in the ovaries of all animals in which there is cyclic growth in the genital tract while the ova are ripening.

Our first tests of material from hens were of extracts made by Dr. Doisy of fresh eggs from the market. These tests returned several negative results and only one questionable positive result. We have since run five distinct series of tests upon hen's ovaries, follicles of different sizes, and mature eggs are listed in Table I, experiments 1 to 5.

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[1] Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxi, 819.

[2] Allen, Doisy, Francis, Gibson, Robertson, Colgate, Kountz, and Johnston, *Am. J. Physiol.*, 68, 138; *Am. J. Anat.*, (in press).

TABLE I.

The source of the follicular hormone of the hen ovary.

Material extracted	Volume of material	Volume of alcohol	Volume of oil	Number of injections	Results
Exp. 1. Follicles	cc.	cc.	cc.		
Small	54	270	6	3	+
Medium	84	420	6	3	—
Large	93	558	6	3	—
Exp. 2. Follicles					
Small	180	720	6	3	+
Medium	55	220	6	3	+
Large, and eggs from tube	180	720	6	3	—
Exp. 3. Follicles					
Small	200	800	7	3	+
Medium	175	700	7	3	+
Large	200	800	7	3	—
Exp. 4. Follicles					
Small	140	280	10	3	+
Medium	420	1680	10	3	+short
Large	340	1360	10	3	—
Exp. 5. Fresh eggs					
18 yolks		1200	7	3	—
24 whites		1300	7	3	—
Exp. 6. Chick embryos, incubated 5 days.					
20, with membranes	33	130	8	3	—
20 whites	190	570	8	3	—
Exp. 7. Chick embryos, incubated 10 days.					
10, with membranes	85	340	7	3	—
10 yolks	130	520	7	3	—
10 whites	110	440	7	3	—
Exp. 8. Chick embryos, incubated 14 days.					
6 with membranes	100	400	7	3	—
6 yolks and whites	75	300	7	3	—

The procedure of preparing and testing extracts was as follows: (1) the ovaries from laying hens were torn from the dorsal body wall and the larger follicles clipped off and separated into classes according to size (average diameter); (a) small follicles, less than 15 mm., (b) medium sized, 15 to 35 mm., and (c) large, 35 mm. to full sized yolks. A few eggs taken from the oviduct were also included in the latter class. (2) These classes of follicles were extracted separately with 95 per cent alcohol for 2 to 4 days, the alcoholic extract filtered off and evaporated, and the residue dissolved in Mazola oil. This product, although crude chemically,[3] is satisfactory for tracing the hormone to its source. (3) The test animals, spayed rats, were given three 1 cc. injections of the oil preparation at 3 to 4 hour intervals. (4) The course of the experiment was followed by microscopic examination of smears of the vaginal contents as described in earlier papers.[4] The details of a typical test are given in Table II.

TABLE II.

A typical test of extracts; experiment 3 as listed in Table I.

Animal	Extract of follicles	Control smear 4/7 3 P. M.	Injections 4/8			Test smears 4/10			Results
			10 A. M.	2 P. M.	5 P. M.	10 A. M.	4 P. M.	4 P. M.	
5	Less than 15 mm.	D	1 cc.	1 cc.	1 cc.	Ea O	O	M_2-D	+
5-NL	15 to 35 mm.	D	1 cc.	1 cc.	1 cc.	O	O-M_1	M_2	+
4-NR	Larger than 35 mm.	D	1 cc.	1 cc.	1 cc.	D	D	D	—

D, operative diestrum; smear of leucocytes indicating absence of growth and slow atrophy of the vaginal epithelium.

O, estrous; smear of cornified epithelial scales indicating that growth of a thick estrous vaginal wall has been induced, (Ea, early phase of this period).

M_1 and M_2 metestrum; degenerative period during which removal of tissue resulting from hormonal action is accomplished by leucocytic infiltration.

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[3] Doisy, E. A., Allen, E., Rolls, and Johnston, *J. Biol. Chem.*, 1924, lix, 43.

[4] Allen, E., *Am. J. Anat.*, 1922, xxx, 297; 1923, xxxii, 293.

Leucocytes were the chief consituents of the control smears on the day before injections were begun (stage D, column 3, Table II), thus checking the condition of operative diestrum or period of complete absence of growth in the vagina of the test animal. Three injections were given on the following day, April 8. As it usually requires 40 to 48 hours for the full reaction of the tissues of the genital tract to the growth stimulus of the hormone,[2] the first test smear was made on the morning of April 10 (column 7). Cornified epithelial cells made up the smears of animals 5 and 5-NL, indicating that full estrous growth or the addition of 8 to 12 layers plus the stratum corneum had been induced in the vaginal epithelium (stage 0, column 7, Table II). The smear of animal 4-NR was composed chiefly of leucocytes, indicating a negative result. Smears made at 4:30 P. M. of the same day confirmed the results of the morning examination. These details of the tests are more fully described and illustrated in earlier papers. The results of these series of tests are listed in the last column in Tables I and II.

The fifth series in Table I shows negative results obtained from extracts of whites of 24 eggs, and yolks of 18; these being fresh fertile eggs from the poultry department of the university.

We have also tested extracts of chick embryos and their membranes, and the whites and yolks, at five, ten and fourteen days of incubation with negative results (experiments 6, 7 and 8, Table I).

Histological examination of the walls of the follicles which contain the hormone show them to have from 1 to 4 or 6 layers of follicle cells in which mitotic proliferation is usually active. The absence of any accumulated secretion equivalent to the liquor folliculi of the mammalian follicle shows that the liquor is not necessarily a fundamental factor in the secretion of the follicular hormone.

To summarize briefly: (1) The ovarian follicular hormone can be extracted from ovaries of laying hens. (2) Injected into spayed rats it causes estrous growth in the genital tract. (3) Extracts of full sized follicles, eggs from the oviduct, fresh eggs (also whites and yolks separately), and 5, 10 and 14 day embryos with their membranes have given negative results. (4) These experiments establish the expected non-specificity of the follicular hormone as concerns birds and mammals.

252 (2484)

A note on the prevention of experimental low-phosphorus rickets in rats by the subcutaneous administration of potassium phosphate.

By ALWIN M. PAPPENHEIMER.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

It is now well established that rachitic lesions of the bones may be readily produced in rats by a regimen deficient in available phosphate. With the standard Diet 84 in use in this laboratory, the minimal protective addition of K_2HPO_4, in the absence of effective light rays or specific antirachitic substances is in the neighborhood of 0.4 grams per cent of the diet—an amount equivalent to 75 mg. of phosphorus. The addition of 50 mg. protects in some individuals, but is on the border line.[1]

It seemed of interest to ascertain whether the subcutaneous administration of inorganic phosphate also conferred protection; and if so, to make at least a rough quantitative comparison of the amount required with that necessary when administered with the food.

A solution of K_2HPO_4 was made up to contain approximately 5 mg. of phosphorus per cc. Actual analysis by the Briggs' modification of the Bell-Doisy method showed the solution to contain 4.85 mg. per cc.[2] The solution was sterilized, and kept in stoppered vaccine bottles to prevent evaporation. Graded amounts were injected 6 days a week over a period of 28 days. There was no local reaction, and the fluid was rapidly absorbed. Two litters of known stock (Gies) comprising 14 individuals, were used for the experiment. Three rats died before the termination of the experiment, and are not included.

The dosage and results are shown in the accompanying table.

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[1] Pappenheimer, A. M., McCann, G. F., and Zucker, T. F., *J. Exp. Med.*, 1922, xxxv, 421.

[2] I am indebted to Miss Margaret B. Gutman for carrying out the analysis.

Rat No.	Litter No.	Diet	Days on diet	Amount of K_2HPO_4 solution injected (1 cc. = 4.85 mg. of P)	Total amount of P injected	Initial weight	Final weight	Rickets		X-ray
								Gross	Microscopic	
					mg.	gm.	gm.			
9012	C 5	84	28	—	—	45	50	+++	++++	++++
9013	C 5	84	28	—	—	46	50	++++	++++	++++
9014	C 5	84	28	1.0 cc.	111.55	62	74	normal	normal	—
9015	C 5	84	28	0.8	89.24	52	?	normal	+	±
9016	C 5	84	28	0.6	66.93	60	64	±	+	+
9017	C 5	84	28	0.6	66.93	55	60	+	++	+
9018	C 5	84	28	0.4	44.62	50	58	++	++	++ healing
9019	C 5	84	28	0.2	22.31	42	48	++++	+++	++++
9021	C 6	84	28	—	—	43	44	++++	++++	++++
9023	C 6	84	28	0.6	66.93	50	56	±	++	+
9025	C 6	84	28	0.2	22.31	42	50	+++	+++	++++

It is seen that complete protection was afforded by injections of 1 cc. of the solution, equivalent to an average daily dose of 3.98 mg. of phosphorus. The total amount administered during the 28 day period of the experiment was 111.55 mg. Injection of lesser amounts gave only incomplete protection.

Data as to the phosphorus retention of rats on Diet 84, with and without cod liver oil or light treatment, are available from the published analyses of McCann and Barnett.[3] Their table shows that the mean total phosphorus content of the bodies of four untreated rachitic rats, was 203 mg.; and of 5 treated rats on the same diet, 306 mg. There was thus, under the influence of these prophylactic agents, an additional total retention of 103 mg. of phosphorus, or an average daily retention of 3.7 mg.

This figure corresponds in an interesting way with the above figure of 3.9 mg., needed to afford protection by subcutaneous injection.

The average daily food consumption of two rats (Nos. 111 and 116) on Diet 84 plus 0.4 per cent K_2HPO_4, was found to be 8.94 and 8.86 gm. respectively. This corresponds to an approximate daily intake of 6.7 mg. of added phosphorus—an amount which may be taken as the minimal protective dose per cc. If this estimate holds true for a larger series, one may consider the protective *subcutaneous* dose as approximately 60 per cent of the amount required when administered with the food.

253 (2485)

A comparative cytological study of the neoplasms of animals and plants.

By MICHAEL LEVINE.

[*From the Cancer Division Laboratory, Montefiore Hospital, New York City.*]

The attempt to compare animal cancer and plant overgrowths must involve a cytological study of their respective tissues. For animal cancer three cases of epithelioma were mainly studied together with several cases of breast and rectal carcinoma and spindle cell sarcoma. Of the plant cancers, crown gall was the chief subject of my study, while the hyperplastic tissue of the potato wart was also used.

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[3] McCann, G. F., and Barnett, M., *J. Biol. Chem.*, 1922, liv, 203.

The cytology of human cancer has been much studied. The existence of multipolar spindles has been described by many workers on animal cancers. I find that nuclei with innumerable chromosomes cannot be due only to unequal distribution of the chromosomes on the spindle, in division. From my preparations of animal cancer a more plausible interpretation leads to the conclusion that these hyperchromatic cells arise from large multinucleate cells and from cells with two or more lobulate nuclei. The nuclei in these cells with their centrosomes undergo simultaneous division; their nuclear membranes disappear and we thus get the appearance of multipolar spindles.

Lobulate nuclei in cancerous tissue are not evidence of the existence of amitotic division. The lobulate nucleus indicates rather a high metabolic activity of these cells similar to that found in actively growing tissues of animals.

The monaster stages recorded by von Hansemann and others are rare in my preparations and those that occur are suggestive in appearance of a section through a dividing cell in which the chromosomes have not reached the poles nor have the centrosomes disappeared, yet the constriction for cell division has been almost completed.

Hyperplastic cells are found in crown galls and potato wart. Binucleate and quadrinucleate cells appear in crown gall tissue. No multipolar spindles have been found in my preparations. In plant tumors I have found nothing comparable to the hyperchromatic and hypochromatic cells of Klebs, von Hansemann, Farmer, and others. No true multinucleate anaplastic giant cells appear although large uninucleate and occasionally binucleate cells are found in crown galls. Nuclei with simple constriction on one surface are found in crown gall cells quite commonly. Prankard, and Beer and Arber, and others have found binucleate cells with lobed nuclei in normal tissues of a great number of plants. My preparations show that the mitotic divisions in tissues of crown gall and potato wart are normal in character and not unlike the divisions of young normal growing tissues.

It appears that the abnormality in neoplastic diseases of plants is found in the rate of division rather than the character of the division. The rapid increase of cells is a response to the parasitic stimulation. In animal cancer both the rate and character of the divisions are aberrant.

This paper was read, in Boston, before the Botanical Society of America in December, 1922. A full report with three plates of figures will appear shortly.

254 (2486)

Further studies with the Dick test and active immunization with scarlet fever streptococcus toxin.

By ABRAHAM ZINGHER.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

Further studies have been made with the Dick test along the following lines:

1. Results with the test among special groups of children and adults.
2. Study of the control test with heated toxin and with toxin neutralized with convalescent serum.
3. Study of scarlet fever cases during convalescence.
4. Study of toxins produced by special strains of hemolytic streptococci.
5. Retest of a group of children actively immunized with increasing doses of scarlet fever streptococcus toxin.

1. The application of the Dick test to a group of 320 children at the Horace Mann School and at the Riverdale Country School, New York City, has shown a very high percentage of susceptible individuals in this class of children coming from the more well-to-do homes. The test showed that of 320 children varying in age from 5 to 18 years, 266 or 83.0 per cent were susceptible and gave a positive reaction. The Dick test was also given to 80 nurses at St. Vincent's Hospital, and of these 42 or 52.5 per cent showed a positive reaction. The nurses at this

hospital had been previously tested with the Schick test and 55 per cent were found susceptible to diphtheria. A group of babies were tested at the Heckscher Foundation with the following interesting results:

Age	Total tested	Dick positive	Dick negative	Per cent Dick positive
0-3 mo.	20	1	19	0.5
3-6 mo.	17	9	8	52.7
6-9 mo.	4	4	0	100.0
9-12 mo.	7	7	0	100.0
1-5 yrs.	25	20	5	80.0
Total	73	41	32	56.1

Of a group of 4570 individuals varying in age from a few weeks to over 20 years we found that 1543 or 34.4 per cent gave a positive Dick reaction. Up to 6 months the percentage of positive reactions was 44.8; from 6 months to 3 years it ranged from 64 to 71 per cent; from 3 to 5 years between 46 to 56 per cent and from 5 to 20 years between 24 to 37 per cent. In those over 20 years of age there were 18 per cent positive reactions.

2. Further study with the control in the Dick test has shown that the best solution to be used for this purpose is toxin which has been heated in its final dilution in a water bath at boiling temperature for one hour. A control consisting of neutralized toxin has one distinct disadvantage. The convalescent serum added to the toxin neutralizes not only the soluble toxin but also the action of the streptococcus protein which produces pseudo and combined reactions in certain individuals. These persons would therefore give positive reactions instead of pseudo or combined reactions. In some individuals the inhibition of the protein action on the skin in the control test is only temporary, the reaction there appearing in pseudo and combined reactors after 48 hours.

3. The routine application of the Dick test in scarlet fever convalescent patients has shown that the large majority give a negative Dick reaction after the 10th day of illness. There are a few patients, however, who give a positive reaction throughout convalescence. Some of these patients show desquamation, others do not. The question arises whether those who do not desquamate have had scarlet fever. The continued positive reaction in some of the patients who show desquamation indicates that there may be more than one toxin produced by the hemolytic

streptococci associated with scarlet fever. If only a single toxin were produced by the different strains of the scarlatinal hemolytic streptococcus only one type of antitoxic antibodies would develop during the later stages of the disease and all patients would show a negative Dick reaction to the same toxin preparation. Another point that has to be considered is the important question whether we are not dealing in scarlet fever with a variety of clinical entities which are classified and grouped as scarlet fever because of the absence up to the present time of any definite criteria except the symptomatic clinical ones.

The following two clinical cases are illustrative:

One patient, a girl 12 years of age, admitted to the Willard Parker Hospital with the history of 10 days of scarlet fever gave a strongly positive Dick reaction on the 12th and 13th days of the supposed illness, at a time when in convalescence from true scarlet fever the Dick reaction is, as a rule, either faintly positive or negative. On the 15th day of the supposed illness, 5 days after admission to the hospital, the patient developed a typical attack of scarlet. The Dick test in this patient so late in the disease indicated that she probably did not have scarlet fever before admission to the hospital.

Another patient, a girl 3 years of age, gave a strongly positive Dick reaction up to the very last day of her stay in the hospital. It is possible that the patient did not have scarlet fever. There was no desquamation, yet the patient did not develop scarlet fever during her stay in the hospital. By analogy with what is well known in diphtheria, one can assume that in the absence of a general immunity to an infectious disease, there is present in the normal mucous membrane of the nasopharynx a local resistance to infection which can be overcome by trauma, as after an operation on tonsils and adenoids, or by an intercurrent inflammation like a cold. The following case illustrates this point. One of the physicians at the Willard Parker Hospital was on duty in the scarlet fever pavilion for 3 months without contracting scarlet fever. When assigned to duty in the diphtheria pavilion he developed diphtheria and 2 days later scarlet fever. The diphtheria process evidently destroyed the local resistance to infection by the hemolytic streptococci, which were probably present for some time but could not exert their pathogenic action on the normal mucous membrane.

An interesting and significant appearance was noted at the site of the original Dick test in two patients who developed scarlet fever one week after the test. The area on the forearm corresponding to the previous reaction was very pale and surrounded by a sharply defined ring of the scarlet fever rash which was greatly intensified in redness when compared with the rest of the eruption on the forearm. During the height of the eruption a second Dick test was made over the pale area and another one a little to one side. The reaction which developed within the pale area was very slight when compared with the second reaction alongside of it. Apparently a certain amount of cellular immunity had developed at the site of the original positive Dick test. This gave rise to the appearance of the local pallor, which presented such a striking contrast to the rest of the blushed skin and also prevented the development of a good positive reaction during the first day of the disease. The ring of intensified rash making up the border which surrounded the pale area was probably due to the interaction between cells which were sensitized rather than protected by the minute antibody content within their substance and the scarlet fever toxin of the disease. These observations point to the probable local origin in the epidermis of some antitoxin production against scarlet fever.

4. Toxins produced by different strains of hemolytic streptococci, isolated from a variety of sources by Dr. Anna W. Williams and her associates at the Research Laboratory were studied for their local action on the human skin and their neutralization by scarlet fever convalescent serum. These toxins were produced by Dr. Williams according to the method suggested by Dick and Dick. A number of strains were thus investigated. Of these there were four strains, one from a case of osteomyelitis, one from a wound, one from a throat culture from a case of measles, and one from a normal throat, which were found to produce a toxin that could be neutralized by convalescent serum.

5. A group of 40 Dick positive children injected in an institution two months ago with 10, 25, 100 and 250 skin test doses of scarlet fever streptococcus toxin at weekly intervals gave the following results at the Dick retest made one month after the last injection: 18 of the 40 children or 45.0 per cent gave a negative reaction; 10 or 25.0 per cent gave a positive reac-

tion which was less marked than the original one and 12 or 30.0 per cent a positive reaction similar to the original one.

A second group of 143 Dick positive children injected in another institution and retested one month later showed that 104 or 72.7 per cent had become immunized; of these, 98 or 94.6 per cent gave a pseudo reaction. Of the 39 who were still positive 28 or 71.7 per cent had a combined reaction. This large number of pseudo reactors at the retest indicates that a certain amount of protein sensitiveness develops after the toxin injections. The toxin used in this work was kindly supplied to me by Dick and Dick. Purification and concentration of the toxin will eliminate most of these proteins. A similar increased protein sensitiveness I have found with the Schick retest after injections of 1/10 L+ mixtures of diphtheria toxin antitoxin.

The local and constitution reactions following immunizing injections of relative small doses of toxin vary with different individuals and depend upon the varying susceptibility of these individuals to the toxin and the protein of the toxic fluid. In a group of 30 children between 5 months and 5 years of age injected at the Heckscher Foundation with the initial dose of 100 skin test doses, 6 had slight temperature, varying between 99.6 and 100.2 and showed a diffuse scarlatiniform rash the following day. The rash lasted 24 to 48 hours. After a second injection of 250 skin test doses given a week later no local reaction at site of injection and no rash were noted. Our experience has been that, as with the diphtheria toxin-antitoxin, the younger children show much less pronounced local and constitutional reactions after injections of scarlet fever streptococcus toxin than do older children and adults.

Up to the present time we have injected with the toxin over 1000 positive Dick reactors. The dosage is as follows: For children under 12 years we give 100, 250 and 250 skin test doses; for children over 12 years, 100, 250 and 500 skin test doses. For adults the last dose could be increased to 1000 skin test doses. A dilution of the toxin is made to contain 500 skin test doses per cc. 0.2 cc., 0.5 cc. and 0.5 cc. is used for children under 12 years, and 0.2 cc., 0.5 cc. and 1.0 cc. for children over 12 years of age. The injections are given subcutaneously or intramuscularly about the middle of the outer surface of the arm and repeated at intervals of 7 days.

255 (2487)

Properties of purified Dick scarlatinal toxin.

By F. M. HUNTOON.*

[*From the Biological Laboratories, H. K. Mulford Company, Glenolden, Pa.*]

The Dick scarlatinal toxin is the filtrate from the growth in broth of certain streptococci isolated from the throats of scarlet fever cases, as has been shown by the Dick's, Dochez and Sherman.

The toxic substance is produced in the largest quantities after the addition of considerable amounts of horse blood.

The best toxin in our hands has been produced by 7 day growths. It has been found possible to purify such toxins to a very considerable degree. If the material is treated with ammonium sulfate up to 60 per cent saturation, with the addition of 1 per cent acetic acid, a voluminous precipitate occurs, taking with it very little of the toxin. On the addition of more ammonium sulfate, no more precipitate occurs until around 70 per cent saturation. This increases up to about 75 per cent saturation. This precipitate carries with it the toxic substance. Such a precipitate is collected and redissolved in distilled water with the addition of sufficient NaOH to neutralize the acid present and is then dialyzed until free of salt. A product is thus produced which contains three quarters of the original toxicity as measured by the skin test, and contains so little nitrogen as to be within the realm of experimental error.

The second method of purification consists in the addition of 20 per cent sodium chloride, 1 per cent acetic acid, filtering and saving the filtrate. This filtrate is then dialyzed, during which time it about doubles in volume. This is found to have about one half the original toxicity and with the nitrogen content of 50 mg. per hundred cc. as contrasted to 850 mg. per hundred cc. in the original material.

Material purified by the ammonium sulfate method and at the same time concentrated so that a skin test was positive with

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* Introduced by T. S. Githens.

1/20 of a cc. of a 1:6000 dilution, was trypsinized by the addition of 1:1000 Fairchilds Trypsin in 2 per cent sodium bicarbonate.

A control of the toxin with bicarbonate present, was kept under the same conditions as was the trypsin control, with no toxin present. After 24 hours these were tested at a dilution of 1:5000 and gave no reaction with the trypsin lot; a plus reaction with the toxin control; and a minus with the trypsin control. These tests were repeated after 4 days, using a stronger dilution of the trypsin lot, with a similar result, so that it may be stated that the toxin is affected by trypsin.

Purified material, which gave a strong reaction in 1:1000, when heated to 90° for 1 hour in a dilution of 1:500, gave no reaction at all, even when tested on those sensitive to horse proteins. A dilution of 1:500, mixed with convalescent scarlet fever serum in equal proportions, incubated for 30 minutes at 37° C., and injected into the skin, gave no reaction.

The toxic substance is totally insoluble in acetone, absolute alcohol, and ether, nor is it inactivated by these materials. Therefore it is not of a lipoidal nature.

CONCLUSIONS.

The toxic substance in the Dick toxin is not of the nature of a globulin, but is precipitated with the higher albumin fractions.

It is a protein substance which is destroyed or inactivated by trypsin.

It is inactivated by heat (90° for 1 hour).

Its tendency to come down in a narrow range of ammonium sulfate precipitation renders a high degree of purification possible. This, in the preparation of material for testing humans for susceptibility, makes it possible to produce solutions with a minimum tendency to produce pseudo reactions.

Lastly, the material is neutralized by convalescent serum, but is not neutralized by normal horse serum or by the serum of horses immunized against the scarlet fever streptococcus in the ordinary manner (non toxic emulsions).

256 (2488)

Studies on acetol as an intermediary product in carbohydrate metabolism.

By WILLIAM S. COLLENS and H. J. DEUEL, Jr.

[*From the Physiological Laboratory of Cornell University Medical College, New York City.*]

In view of the fact that it has been demonstrated that acetol results in small amounts from the distillation of a large variety of the simpler carbohydrates with weak sodium bicarbonate solution,[1] an investigation was undertaken to determine whether such a change might not be a normal physiological process.

Greer, Witzemann, and Woodyatt[2] fed 10 and 20 gm. doses of acetol to dogs and in three experiments could demonstrate no extra glucose, and believed there was some evidence that this substance was partially converted to the acetone bodies. Acetol was made by the method outlined by Nef.[3] In the first series of experiments it was fed to a normal dog together with *o*-aminobenzaldehyde in order to determine whether the combination between acetol and *o*-aminobenzaldehyde into a readily detectable fluorescent 3-oxyquinaldine would take place *in vivo* as it does *in vitro*.[4] Since this combination could not be brought about in the animal organism, it was impossible by feeding *o*-aminobenzaldehyde to demonstrate the presence of acetol normally in the dog.

In the second set of experiments acetol was fed to the completely phlorhizinized dog on the fourth or fifth day of phlorhizination and the amount of any extra production of sugar was determined by calculations based on the prevailing D:N ratio. In the protocols given below only the results of the analyses from the fourth day are given. Urinary nitrogen was determined by the Kjeldahl method, glucose by the Benedict method, and total acetone bodies by the procedure of Van Slyke. In order to

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[1] Baudisch, O., and Deuel, H. J., Jr., *J. Am. Chem. Soc.*, 1922, xliv, 1585.

[2] Greer, J. R., Witzemann, E. J., and Woodyatt, R. T., *J. Biol. Chem.*, 1913-14, xvi, 455.

[3] Nef, *Ann. d. Chem.*, 1904, ccclxv, 260.

[4] Baudisch, O., *Biochem. Z.*, 1918, lxxxix, 279.

Dog	Date	Catheterization	Phlorhizin	Urine							Remarks.
				Vol.	Dextrose	Nitrogen	Extra glucose	D : N	Acetone bodies	Acetone per hr.	
			gm.	cc.	gm.	gm.	gm.		gm.	gm.	
1	Feb. 1	9:45	1								
	Feb. 2	9:45	1	610	22.36	6.86		3.25			10 gm. acetol given at 1:45 p. m.
		1:30		160	3.38	0.96		3.53			
		6:35		225	8.81	1.81	2.42	4.86			
		10:35		205	4.73	1.39		3.64			Some vomited.
2	Feb. 21	10:00	1								
	Feb. 22	9:35	1	610	25.59	7.55		3.39	0.554	0.0236	
		2:35		186	5.72	1.76		3.25	0.103	0.0206	10 gm. acetol given at 3:10 p. m.
		7:35		430	12.60	1.79	8.07	7.04	0.186	0.0372	
		12:35		205	5.96	1.44		4.14	0.165	0.0330	
	Feb. 23	9:35	1	545	8.45	2.61		3.24	0.370	0.0411	
		2:35		270	4.34	1.35		3.22	0.246	0.0492	7.06 gm. acetol given at 3:40 p. m.
		7:35		468	4.42	1.32	0.68	3.35	0.137	0.0274	
		12:35		182	4.99	1.39		3.59	0.104	0.0208	
	Feb. 24	9:35		490	8.17	2.44		3.31	0.289	0.0321	
3	Mar. 11	9:55	1								
		12:55		125	2.40	0.93		2.58	0.315	0.1050	10 gm. acetol given at 1:00 p. m.
		4:55		180	4.83	1.30	3.17	3.71	0.286	0.0715	
		8:55		676	4.07	0.92		4.42	0.314	0.0785	
	Mar. 12	12:55		425	4.36	1.27		3.35	0.234	0.0585	
		9:55	1	350	6.81	2.16		3.15	0.235	0.0261	
		12:55		164	2.27	0.26		8.73			Dog shivered.
		3:55		172	2.03	0.56		3.62			10 gm. acetol given at 4:00 p. m.
		7.55		233	1.91	0.53		3.60			
4		Dog previously shivered.									
	April 16	9.10	1								Dog died.
		1:10		225	4.25	1.41		3.01			10 gm. acetol given at 1:10 p. m.
		5:10		365	4.93	1.37	0.20	3.59			
		9:10		240	5.05	1.88		2.70			
		12:10		212	2.60	0.76		3.42			
	April 17	9:10	1	430	8.33	2.69		3.10			10 gm. acetol given at 9:10 a. m.
		1:10		472	4.31	1.24	0.42	3.47			
		4:40		450	3.21	1.05		3.06			

determine whether acetol was excreted unchanged after its administration, the urine was distilled in slightly acid solution and a reduction test made on the distillate, always with negative results.

In the first three experiments, in which the animal was not caused to shiver—to completely rid it of glycogen—during a preliminary period, a marked rise in glucose excretion resulted after the introduction of 10 gm. of acetol, which was not manifest on the administration the following day of 7 gm. of this substance (in dog 2). Moreover, when another animal (dog 4) was caused to shiver on the third day of phlorhizination, by bathing it in ice water followed by a two-hour stay in a cold room, no increase in urinary glucose resulted from the first administration of acetol nor from the ingestion of the same amount on the following day. Albuminuria was not present in three experiments, as found by Greer, Witzemann, and Woodyatt.[2]

No evidence is available to show that this substance gives rise to acetone bodies. Just why it is not converted to glucose is problematical in view of the fact that the phlorhizinized dog converts into sugar many closely related substances.

257 (2489)

Some results obtained by the treatment of nervous phenomena with glucose.

By JULIA T. PARKER and CAROLINE S. FINLEY.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

Recently Edwards, Page and Brown[1] have described cardiovascular disturbances accompanying insulin hypoglycemia in dogs. They found that insulin brought about certain irregularities in heart action, a slight decline in mean blood pressure and a lowered CO_2 capacity of the blood. Similar results associated with nervousness have been observed following the injection of insulin in normal individuals or an overdose of insulin in diabetics, by Banting and his associates.[2, 3, 4] These results are apparently due to lowered blood sugar and may be relieved by the administration of glucose or of adrenalin. This action of adrenalin is attributed to its glycogenolytic effect on the liver.

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[1] Edwards, J. J., Page, I. H., and Brown, P. K., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 170.

We were impressed by the similarity of these findings in insulin hypoglycemia to those in some young patients with low blood sugar and low CO_2 capacity who were suffering from severe nervous manifestations. Experiments were conducted in the feeding of glucose to these children, with immediate and striking relief of their symptoms. This encouraged us to try glucose therapy in a number of other cases with similar but less severe symptoms with like success.

The symptoms in the cases referred to were either psychic—asthenia, anorexia, phobias, etc.—or somatic symptoms such as tachycardia, palpitation and digestive disturbances, commonly thought to be psychogenic. These were associated with a blood sugar, which in most instances was within the limit generally considered normal, but below the average finding, a low CO_2 capacity of the blood and a high red cell count and hemoglobin, which latter findings were in contrast with the anemic appearance of the patients. The significance of the relatively low blood sugar is indicated by the fact that the patients' symptoms were promptly relieved by feeding glucose, and this relief in some instances was associated with a slight increase of blood sugar.

We have summarized the blood findings and clinical symptoms in ten of these cases in the accompanying table. We believe that they represent a group in which symptoms which are apparently psychogenic have a definite metabolic cause.

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[2] Banting, F. G., Campbell, N. R., and Fletcher, A. A., *Brit. Med. J.*, 1923, i, 8.

[3] Fletcher, A. A., and Campbell, N. R., *J. Metabl. Research*, 1922, ii, 637.

[4] Banting, F. G., Best, C. H., Collip, S. B., MacLeod, J. J. R., and Noble, E. C., *Am. J. Physiol.*, 1922, lxii, 162.

Case	Sex	Age	Blood sugar	CO_2 capacity per 100 cc. blood.	Symptoms	Dose of glucose in gm. per day necessary to relieve symptoms
			mg. per 100 cc	cc.		
1	♀	12	85	58	Pallor, emaciation, phobias, irregular pulse.	60 to 100
2	♀	28	78	56	Pallor, emaciation, asthenia.	80
3	♀	28	92	56	Pallor, emaciation, asthenia, asthma.	70
4	♀	11	73	55	Pallor, asthenia.	60
5	♀	23	92	52	Pallor, emaciation, asthenia, night terrors, indigestion.	50
6	♀	60	87	61	Pallor, emaciation, asthenia, phobias, irregular pulse, indigestion.	50
7	♀	35	75	56	Pallor, asthenia, irregular pulse, indigestion.	60
8	♀	14	85	58	Pallor, phobias, night terrors, irregular pulse.	50
9	♂	21	90	60	Pallor, emaciation, asthenia.	50
10	♀	55	86	50	Pallor, asthenia, neuralgic pains.	40

258 (2490)

Cyclic variations in the spontaneous contractions of the human fallopian tube.

By DANIEL L. SECKINGER and FRANKLIN F. SNYDER.*

[*From the Carnegie Embryological Laboratory, The Johns Hopkins Medical School, Baltimore, Md.*]

In two recent papers it has been shown that periodic variations occur in the spontaneous contractions of the Fallopian tube during the reproductive cycle in the pig (Seckinger, 1923)[1] and in Macacus rhesus (Corner and Seckinger, 1923).[2] These investigations demonstrated that there is a characteristic type of contraction at the time of ovulation in both the pig and the monkey. With a knowledge of this work, we have attempted to investigate the tubal contractions in the human during the different phases of the menstrual cycle and gestation.

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* Introduced by George B. Wislocki.

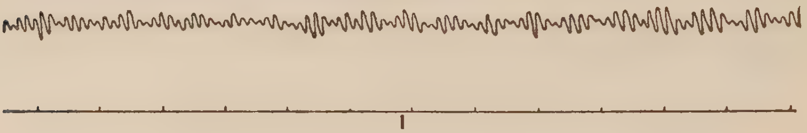
[1] Seckinger, D. L., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 236.

[2] Corner, G. W., Seckinger, D. L., *Anat. Record*, 1923, xxvi, 299.

Our method was similar to that used by Seckinger and Corner. Rings of Fallopian tube, immersed in warm, oxygenated Locke solution, were suspended from a lever which recorded on a kymograph. Our observations are based upon records of more than forty Fallopian tubes obtained from the Woman's clinic of the Johns Hopkins Hospital, through the courtesy of Dr. Cullen and Dr. Williams. Specimens which showed marked pathological changes were rejected.

We found in our series two types of tubal contractions. In one type the contractions were slow (about 4 per minute) and of equal amplitude (Figs. 2, 3), while in the other they were more rapid (about 8 per minute) with a tendency to periodic alternation in amplitude (Fig. 1). Transitions were found between these two characteristic types.

An attempt was made to correlate these striking physiological variations with the coincident histological changes observed in the uterus, tubes and ovaries of the same specimens. It was found as a rule that the slow contractions of equal amplitude occurred throughout pregnancy and the premenstrual, menstrual and early interval phases of the cycle. Figure 3 illustrates the contractions of a tube from pregnancy. Figure 2 is of a speci-



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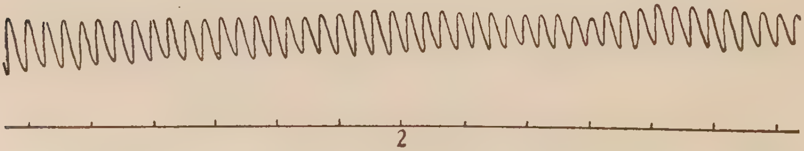

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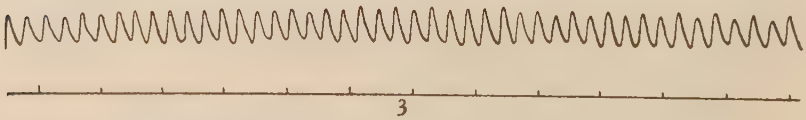

3

men from the menstrual phase. The more rapid contractions of unequal amplitude were observed during the midinterval and late interval phases (Fig. 1). The latter type of contraction is very similar to the type described by Seckinger and Corner as characteristic of the time of ovulation in the sow and monkey.

It may be of interest to describe the ovary of the specimen from which the tracing shown in Figure 1 was obtained. This ovary showed a dark purplish mass containing a cavity filled with a recent blood clot. On section the wall of the mass showed the granulosa and the theca interna not unlike a very early corpus luteum. This presumably represented a recently ruptured follicle. The Fallopian tube of this specimen showed the smooth, high (35 micra) epithelium characteristic of the midinterval phase in the human (Snyder, 1924).[3] The character of the tubal epithelium and of the ovary, as well as the physiological activity of the tube made it seem very probable that the specimen was from the period of midinterval, shortly after ovulation. Moreover, the clinical history placed the specimen as 14 days after the onset of menstruation.

The detailed observations upon which this summary is based are to appear later.

259 **(2491)**

Spontaneous interstitial myocarditis in rabbits.

By C. PHILIP MILLER, Jr.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

This study began with the observation of myocardial lesions in the hearts of normal rabbits and guinea pigs which served as controls in a series of experiments in which attempts were made to transmit rheumatic fever to laboratory animals.

The rabbits which form the basis of this report were obtained from a variety of sources over a period of 3½ years. They were kept under careful observation for periods varying from a few days to 4 months. None has been included which manifested any clinical sign of disease, such as snuffles, diarrhea, ear canker, loss in weight, cutaneous or subcutaneous infections. They were killed by a blow on the back of the head. A complete autopsy was performed, but only the hearts were prepared for microscopic examination. A number of sections of each heart were examined.

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[3] Snyder, F. F., *Johns Hopkins Hosp. Bull.*, 1924 (in press).

The lesions were somewhat more common in old than in young rabbits. They varied in size from small groups of 6 or 8 cells to large areas the size of a high power field. They consisted of infiltrations between the muscle fibers of lymphocytes and endothelial leucocytes. Some of the lesions, particularly the smaller ones, contained only cells of one type. In others they were mixed in varying proportions, to which were occasionally added small numbers of polymorphonuclear eosinophiles, fibroblasts and plasma cells. The lesions occurred most frequently between the muscle fibers of the papillary muscles and ventricular walls, but occasionally beneath the endocardium and epicardium. None was ever found in the valves or at the bases of the valves. The muscle fibers passing through the lesions occasionally showed loss of striation, but rarely evidence of marked degenerative changes.

Careful search has revealed no bacterial or protozoan microorganisms or cell inclusion bodies. The incidence of the myocardial lesions bore no relation to the occurrence of coccidiosis.

The examination of the hearts of 34 apparently healthy adult rabbits has disclosed the presence of a spontaneous focal interstitial myocarditis in 60 per cent of the animals. This observation assumes considerable interest when it is recalled that several workers have considered the production of experimental myocarditis as one of the important criteria of successful transmission of rheumatic fever to this species of laboratory animal.

260 (2492)

Increased gastrointestinal permeability as a possible factor in parathyroid tetany.

By ARNO B. LUCKHARDT and EDWARD L. COMPERE.

[*From the Hull Physiological Laboratory, The University of Chicago, Chicago, Ill.*]

It is generally conceded that young mammals develop parathyroid tetany sooner and in severer form than adults of the same species. Starvation of any animal prior to and following a complete thyroparathyroidectomy delays the onset of tetany, diminishes its severity, and prolongs life.

There is some evidence that the gastrointestinal tract of the young is more permeable than that of adult animals. The disastrous effect of a meat diet following a parathyroidectomy has been known for a long time and has directed attention to the possibility that toxic products of gastrointestinal origin were responsible for the marked depression or convulsive death of parathyroidectomized animals. The work of Dragstedt and Luckhardt support that possibility (see following article for references). Various observers have described pathological changes in the mucosa of the stomach and upper portion of the small intestine of dogs following fatal tetany.

Such considerations suggested the hypothesis that parathyroid tetany might in part be due to an increased permeability of the gastrointestinal tract allowing toxic products of protein origin to pass into the blood stream in larger amounts and at a more rapid rate than under normal conditions. Such an increased permeability would account for the marked depression and convulsive death. The increased permeability is capable of accounting also for the increased elimination of calcium by the gut and the hypocalcemia which apparently exists. Oral administration of calcium salts might under such conditions render the gut less permeable, preventing the absorption of toxic compounds; restore the calcium balance upset by the increased excretion of calcium by the gut; and meet the heavy demands on the body calcium of a high meat (calcium poor) diet.

If complete parathyroidectomy results in an increased permeability of the gut, the recovery reported in previous publications might indicate either an increased tolerance to the toxins or a return to a nearly normal functional activity of a deranged gastrointestinal tract. It might be expected, however, that such animals that had escaped an otherwise certain death, because of treatment, might be more easily thrown into tetany by any measure designed to injure their gastrointestinal tract and make the latter more permeable.

We accordingly made use of a number of dogs whose life had been observed by either the intravenous injection of Ringer's solution or the oral administration of various forms of calcium salts.[3] These animals were on the usual stock diet or on a daily ration of 1/3 to 1/2 lb. meat and 1/2 lb. bread. As soon as the animals showed no signs of developing tetany spontaneously they were given several cascara pills, 30 to 60 or more grams of sulfur ointment for one or two days, or on other occasions, 3 to 5 minims of croton oil. It was expected that such treatment would set up a more or less intense gastritis and enteritis and by so doing effect a change in the permeability of the gut.

RESULTS.

1. Following the oral administration of a drastic cathartic (croton oil) or the ingestion of moderate quantities of a laxative (cascara or sulfur ointment) dogs that have recovered from the acute effects of a complete parathyroidectomy can be thrown into more or less severe tetany. Marine[4] was "inclined to think that the administration of large amounts of sulfur favors the development of tetany" by "bringing about a state of nutrition favoring the development of tetany" or because possibly some "alkaline earths are withdrawn from the field of absorption by this element."

2. If a slight tendency to tetany exists only small amounts of an irritant are necessary to bring out the symptoms.

3. Such tetany usually develops within the first 24 to 48 hours after administration. Within 24 hours the animals are visibly depressed, show an anorexia, enophthalmos, corneal and conjunctival injection, and some spasticity. More or less severe tetany (clonic or tetanic seizures) may develop in 24 hours but usually occurs in 48 hours.

4. Dogs but recently parathyroidectomized (3 mos.) show

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[3] Compere, E. L., and Luckhardt, A. B., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 526.

[4] Marine, D., *J. Exp. Med.*, 1914, xix, 89.

the symptoms earlier and in severer form than such animals which have survived the removal of their parathyroids from 1 to 2 years. Tetany has not been induced in normal animals by such treatment.

5. The fact, that in several animals tetany, after the administration of croton oil, develops in the absence of catharsis but following repeated emesis of copious amounts of blood stained gastric mucus seems to indicate that the stomach had suffered the greatest injury and that the subsequent tetany may have been due to the absorption of toxic compounds from the stomach (gastric tetany).

6. This fact would indicate that the stomach may under certain conditions possess greater absorptive power than has been accredited to it. In support of this view we cite the further evidence (collected in previous work) that the symptoms of parathyroid tetany become less severe quite *promptly* after emesis of a meal taken 48 *hours previously;* and that necropsy findings of animals dead from tetany often reveal large amounts of food in the stomach with but little material of any kind in the small or large intestine.

7. The development of some of the symptoms of tetany in parathyroidectomized dogs following the administration of monobasic calcium phosphate may be ascribed to its marked irritating action on the gastrointestinal tract.

8. Animals in which tetany has been induced intentionally by a drastic cathartic can be restored to a normal condition by the administration of calcium salts other than the soluble phosphate (*i. e.,* by the acetate, nitrate, carbonate, lactate).

At present we are inclined to interpret the above facts as follows:

1. More or less violent gastrointestinal irritants change the permeability of the gut of parathyroidectomized dogs and permit (a) the more rapid and complete absorption of toxic compounds from the stomach and intestines; and (b) of the more rapid excretion of calcium compounds.

2. The administration of calcium compounds may conserve the life of recently parathyroidectomized animals or those thrown into tetany by gut irritants by restoring the normal impermeability of the gut and supplying the blood and tissues with calcium lost by the increased excretion by the bowel due to an increased permeability.

CONCLUSION.

In a previous publication we have called attention to the possibility that our completely parathyroidectomized animals may on occasion show convulsive seizures indistinguishable from grand mal attacks of idiopathic epilepsy seen in man. The same factors which predispose to such attacks in man favor the convulsive attacks in such dogs. Indiscretions of diet particularly in infants are likely to induce convulsive seizures with definite clinical indications of a gastritis and enteritis. Whereas it is desirable to prevent constipation in epileptics and promote intestinal evacuation following any dietary indiscretion, it is obvious, on the basis of the results just described, that too drastic catharsis may be more harmful than beneficial in that any violent cathartic may for reasons advanced rather induce convulsive seizures than prevent them.

261 (2493)

On the efficacy of various calcium salts in parathyroid tetany.

By EDWARD L. COMPERE and ARNO B. LUCKHARDT.

[*From the Hull Physiological Laboratory, The University of Chicago, Chicago, Ill.*]

Several satisfactory methods are now available to preserve the life of dogs following an otherwise rapidly fatal thyroparathyroidectomy. Forced diuresis by the intravenous injection of large quantities of Ringer's solution quite rapidly controls the symptoms of parathyroid tetany, or if maintained in the absence of any symptoms even prevents it appearance.[1] The oral administration of calcium lactate[2] is less laborious and even more effective. If, however, the animal at any time develops *severe* tetany in spite of treatment the intravenous injection of Ringer's solution is indicated as the first measure of choice to obviate the extreme tetany with exitus possibly attendant on the passage of the stomach tube. Under any condition the oral administration of calcium lactate is safer and more physiological than its intravenous injection[3] and the literature bears evidence of our contention that many dogs in tetany have probably died, not in spite of such intravenous injections, but probably because of them. The dietary method of conserving the life of parathyroidectomized dogs devised by Dragstedt[4] and used by him in a number of studies involves the use of milk (and bread) enriched with large quantities of lactose. Under this regime the intestinal flora was kept aciduric due partly to the high lactic acid content of the intestinal material as well as to the altered metabolism of the bacteria in the presence of an abundant source of available carbohydrate and absence of large amounts of animal protein (meat) from which under the usual conditions, the bacteria might and do form the toxic compounds responsible for the tetany.

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[1] Luckhardt and Rosenbloom, PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 129; Luckhardt and Rosenbloom, *Science*, 1922, lvi, 48; Luckhardt and Blumenstock, *Science*, 1922, lvi, 257.

[2] Luckhardt and Goldberg, *J. Am. Med. Assn.*, 1923, lxxx, 79; Luckhardt and Blumenstock, *Am. J. Physiol.*, 1923, lxiii, Feb. (Proceedings).

In our own studies, meat was allowed plentifully, but when given with large amounts of calcium lactate (1.5 gm. per kg.) maintained the animal in an excellent state of health and nutrition. It seemed possible that the lactate ion, present in high concentration in the bowel, might be in a great measure responsible for the beneficial action of the calcium lactate fed; and that its presence (like the lactose or lactic acid) had so altered the metabolism of the intestinal flora that the toxic compounds responsible for the tetany would not be formed. In spite of the fact that under our regime of intensive calcium lactate feeding the intestinal flora on a high protein intake maintain their essentially gram negative character, we nevertheless tested out the efficacy of other calcium salts when fed in large quantities, using the same technique as described in a previous communication.[2] Finding the chloride unsatisfactory because too irritating, we restricted our studies up to the present to calcium nitrate, calcium acetate, calcium carbonate, and the soluble monobasic calcium phosphate.

RESULTS.

1. Calcium carbonate, calcium nitrate, and calcium acetate

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[3] Blumenstock and Luckhardt, *Am. J. Physiol.*, 1924, lxviii, March (Proc.).

[4] Dragstedt, *J. Am. Med. Assn.*, 1922, lxxix, 1593; *Am. J. Physiol.*, 1923, lxiii, (Proc.); Peacock and Dragstedt, *Am. J. Physiol.*, 1923, lxiv, 499; Dragstedt, Phillips and Sudan, *Am. J. Physiol.*, 1923, lxv, 368; Dragstedt, Phillips and Sudan, *Am. J. Physiol.*, 1923, lxv, 503.

can be administered orally in large quantities (1.5 gm. per kg. daily) to completely parathyroidectomized dogs with no visible deleterious effect.

2. These forms of calcium are as effective as the lactate in preventing by their oral administration the development of tetany or rapidly restoring the animal to its normal condition (in 1½ hours) if tetany has developed spontaneously or has been induced intentionally.

3. Because of their irritating properties to the gastro-intestinal tract the acetate and nitrate are more likely to produce emesis than the lactate or the carbonate.

4. Calcium carbonate, though tasteless (unlike the bitter lactate), is physiologically as efficient but decidely more constipating than the lactate (acetate or nitrate).

5. The soluble calcium phosphate, on the other hand, is not only highly irritating but induces enophthalmos, vomiting, abdominal distress, great depression, hyperpnea, some spasticity, and pronounced salivation. It cannot be used, therefore, in place of the other salts of calcium and will receive further study. Calcium phosphate seems rather to favor the development of tetany than lead to its disappearance.

6. Calcium salts, with the exception just noted, will carry a recently parathyroidectomized but pregnant bitch through pregnancy, labor, and normal lactation although the pregnant and lactating animal is not as easily managed as a normal dog.

262 (2494)

Ketosis associated with conditions of alkalosis.

By LELA E. BOOHER and JOHN A. KILLIAN.

[*From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York City.*]

Abnormally large amounts of acetone bodies have been observed in the blood associated with conditions of uncompensated alkalosis, due either to alkali therapy or to excessive loss of HCl through vomiting. The pH of the blood plasma was determined by the method of Myers, Schmitz and Booher and the acetone bodies by the method of Van Slyke and Fitz. For example, values for acetone bodies of the blood of 60, 50, 66, 28 and 8 mg. per 100 cc. were found associated with pH values of 7.58, 7.54, 7.50, 7.52 and 7.50 respectively. The bicarbonate contents of these bloods were 84, 83, 88, 87, and 70, respectively. The amount of acetone bodies excreted in the urine was determined in two instances and was found to be abnormally large.

263 (2495)

A reaction given by insulin solutions in-vitro.

By STANLEY R. BENEDICT.

[*From the Department of Chemistry, Cornell University Medical College, New York City.*]

It has been found that solutions of insulin (Iletin, Lilly) markedly accelerate the digestion of starch by various diastases. The studies thus far seem to show that this action is not due to the hydrogen ion or to the protein content of the insulin solutions. Further work is, however, necessary to show definitely whether the insulin is the effective factor. The work is being continued and is including a study of the effect of diastase injections upon blood sugar concentration.

ABSTRACTS OF COMMUNICATIONS.

Pacific Coast Branch.

Forty-first meeting.

Berkeley, California, April 12, 1924.

264 (2496)

Electric charges and stability in suspensions of red blood cells.

By JEAN OLIVER and L. BARNARD.

[*From the Department of Pathology of the Medical School of Leland Stanford Junior University, San Francisco, Calif.*]

A quantitative study by means of the Michaelis cataphoresis cell of the electric charge of red cells suspended in isotonic sucrose solution has given the following results:

The negative charge of the cells in pure sucrose solution is not permanent, but falls in the course of a few minutes to a point so near zero that it can not be accurately determined. With this fall in potential agglutination of the suspension occurs.

Electrolytes affect the charge of the cells in the same manner as has been observed for their action on the charge of other suspended particles. All electrolytes depress the original negative charge to an isoelectric point and some reverse this negative charge to positive one. With negative cells the degree of change varies with the valency of the cations present. The observation of the effect of valency is, however, complicated by the fact that the cells act as amphoteric electrolytes with the result that hydrolysis may cause the salt of a bivalent metal ($CuCl_2$) to produce more marked changes than are caused by the salt of a trivalent metal which hydrolyzes less ($CeCl_3$). Reversal of the negative charge to a positive one is produced by all trivalent ions and occurs with bivalent ions of strongly hydrolyzed salts. These positive charges are permanent and so differ from the negative charge which exists in weak concentrations of all electrolytes.

Stability of the suspension occurs whenever the charge, either positive or negative, is above a certain critical potential. But stability may also occur in the complete absence of any potential in high concentrations of all electrolytes. "Irregular series," in which a zone of stability is found between two zones of instability, are produced by a reversed strongly positive charge which surpasses the critical potential. Such an effect is produced by trivalent salts and is also observed with salts of bivalent metals which are strongly hydrolyzed. In the latter case the reversal is probably due to the effect of the H ions acting on the amphoteric red cells. "Prozones," in which a zone of stability in the highest concentrations of the electrolyte is followed by a zone of instability, are not the result of charges of the cells.

A suspension of red cells in sucrose resembles, therefore, a lyophobe colloid in its sensitivity to flocculation by electrolyte, and the mechanism of this flocculation is the same as is observed with such solutions. The stability, except in the very highest concentrations of electrolyte, depends on the potential of the electric double layer at the surface of its suspended particles. On the other hand, both in the chemical nature of its particles and in the amphoteric reaction of these to H and OH ions, it resembles more closely the lyophile colloids, such as proteins and gelatin.

Such solutions do not depend on the charge of their particles for stability, but more likely on "chemical forces acting in true solubility" (Loeb).

This resemblance of a suspension of red cells to a lyophobe colloid casts a certain light on the nature of the suspended cells. The protoplasm of red cells consists of a semi-fluid jelly-like substance of the nature of a lyophile colloid, but the stability of a suspension of suspended particles of such nature would not react to electrolyte flocculation as does a suspension of red cells, whose stability has been shown to depend on electric charges. If, however, it is assumed that the surface of the cells is covered with an insoluble film of amphoteric character, the conditions which obtain in a red cell suspension are met.

Protein, when denatured, loses its lyophile properties so that the stability of a suspension of it depends on the electric properties of its particles, which are also amphoteric in reaction with H and OH ions. Ramsden has shown that many proteins are denatured in film formation, and Loeb has shown that genuine crystaline egg albumin loses its hydrophile properties and acts as a lyophobe colloid when it is deposited in a thin layer on collodion particles. Such protein coated particles resemble very closely a suspension of red cells in their flocculation by electrolytes and in their reaction with H and OH ions.

It is suggested that a denaturing of the proteins on the surface of the cell and the formation of a membrane would account for the properties of the cell suspension. The insolubility of this surface film would cause the stability of the suspension to depend on electric charges; the chemical nature of the film would account for its amphoteric properties.

265 (2497)

A study of the bi-refringence of agar agar.

By JOHN FIELD, 2nd, and C. L. ALSBERG.

[*From the Department of Chemistry and the Food Research Institute, Stanford University, Calif.*]

Many gels when subjected to strain become anisotropic. A film of gelatin or colloid dried on glass is anisotropic with one optical axis normal to the surface of the film.[1] With the removal of the strain as by moistening the film, the anisotropy largely disappears. The writers undertook to determine whether or not in the case of agar agar this property is inherent in the colloid itself or exhibited by it only in the gel state. To this end anisotropy was looked for in agar that had been dried in the gel form and in agar that had been dried in the sol form.

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[1] F. Weigert and H. Pohle, *Kolloid Z.*, 1921, xxviii, 153.

To obtain dried agar gel for examination under the microscope, drops of agar solutions ranging up to one per cent were placed on glass slides and dried in a vacuum desiccator at room temperature.

To obtain agar preparations dried in the sol form, drops of the same solutions were dried in a thermostat at 35°. That the agar agar preparation used in this work exists in the sol form at this temperature appears from a study of the viscosity of the solutions as the temperature is lowered. At various temperatures above 34° there is very little change in the viscosity. At 34° the viscosity begins to increase at an extremely rapid rate. Gelation occurred between 34° and 30°.

Under the polarizing microscope the agar dried as a gel shows very strong bi-refringence. The preparations were decidedly anisotropic. With a selenite plate addition colors were obtained. With a plate of red of the first order placed between the nicols at an angle of 45° to the plane of the nicols, addition colors were obtained, blue when the striæ were parallel to the optical axis and yellow when perpendicular. With the test plate white of the first order, the striæ when parallel to the optical axis were faint yellow and when perpendicular, gray.

The agar dried as a sol showed but very faint bi-refringence, and this was limited to the periphery of the drop. The interior of the drop was free from even this very faint anisotropy. With the selenite plate colors appeared only at the periphery and were very faint but similar to those observed in the gel. When the film was cracked the edges of the cracks showed decided bi-refringence.

That the marked difference in the optical properties of the two forms of agar is not due to difference in the thickness of the layers examined is evidenced by the fact that the sol form showed no anisotropy at all over the greater part of the surface of the film. Whether the bi-refringence of the gel form is as great as above recorded when in drying it is not adherent to a support and hence is permitted to contract uniformly, is being investigated. That the two forms of dried agar have different structure is clear. Whether the removal of strains plays a rôle in the mechanism of the imbibition and swelling of gels, as well as in their behavior in some other respects, is under investigation.

266 (2498)

On the gelatinization by heat of wheat and maize starch.

By C. L. ALSBERG and O. S. RASK.

[*From the Food Research Institute and the Department of Chemistry, Stanford University, Calif.*]

By means of the Stormer viscosimeter it was found that during the gelatinization process the viscosity of wheat and maize starch suspensions in water increases gradually over a range of 25 to 30 degrees. Gelatinization is not a sharp transition point like the melting points of crystalline substances. It resembles more nearly the gradual softening processes of glass and other non-crystalline substances that show no definite melting points. This conception is consistent with the disappearance of anisotropy in the early stages of gelatinization. There is good evidence for the belief that the anisotropy of the starch grain is the result of strain. Heating in the presence of water, it is probable, destroys the anisotropy because it removes the strains. The process is analogous to annealing.

Possibly the gelatinization of starch is to be regarded in this wise: The starch gel first imbibes water. In this process strains are presumably removed and in consequence anisotropy disappears. When the starch gel has thus reached a certain degree of dilution by imbibition, it melts. If this is the course of events, it is not necessary to assume, as has been done so frequently, that the gelatinization of starch depends upon depolymerization. The conception of gelatinization here presented is in accordance with certain observations that are not readily explainable on the assumption that starch during gelatinization depolymerizes—using this term in a strictly chemical sense. These observations are: that sharply drying starch or thoroughly wetting it changes the gelatinization temperature; that gelatinization is incomplete unless adequate quantities of water are present; and that the rate of heating has a marked effect upon the gelatinization temperature.

267 (2499)

A metabolic study of Bacterium solonacearum E. F. S.

By A. P. KRUEGER and C. L. ALSBERG.

[*From the Department of Chemistry and Food Research Institute, Stanford University, Calif.*]

Bacterium solonacearum E. F. S. is the organism causing the brown rot of the tomato, tobacco, and a number of other plants. Among the symptoms that it may produce is a peculiar form of dwarfing which suggests the possibility that this stunting is an intoxication effect. Preliminary to an investigation of this possibility a general study of the organism's metabolism was made. The organism grows slowly on a culture solution consisting of 1 per cent peptone, 5 per cent glucose and tap water. Excess of calcium carbonate does not apparently change its rate of growth. Upon Meyer's Mineral Medium, if the seeding be heavy and with an actively growing culture, the organism grows slowly. It grows very little, if at all, upon plain agar. The optimum reaction seems to be +12, Fuller's Scale. The addition of a minute amount of the aqueous extract of either crushed tomato plant or tomato fruit enormously stimulates growth in all media upon which growth is at all possible. Thus, one of the best media for the organism is Meyer's Mineral Medium enriched with 5 per cent glucose, 1 per cent peptone, and a small amount of either tomato plant or tomato fruit extract that has been neutralized. Grown for two months upon 1 per cent peptone, 5 per cent glucose, and tap water, with and without an excess of calcium carbonate, the following products are produced: Very small quantities of acetic acid and of ethyl alcohol; appreciable quantities of monamine, diamine, triamine, and ammonia. The amines were separated from the main body of the filtered culture medium by volatizing them after the addition of fixed alkali with a current of air as in the Folin method for the determination of ammonia. The determination of the different amines was made by the method of Weber and Wilson. In these cultures but a very small percentage of the glucose originally present had disappeared. No non-volatile acids could be detected.

268 (2500)

Factors influencing the determination of gluten in wheat flours.

By D. B. DILL.*

[*From the Food Research Institute and Department of Chemistry, Stanford University, Calif.*]

The washing of gluten from wheat flour has a two-fold purpose. An approximate idea of protein content is obtained from the weight of dry gluten. The per cent of water imbibed by the gluten together with its coherence and other physical properties furnish the analyst with a rough index to flour strength.

Tap water is usually employed for gluten washing, but this has obvious disadvantages on account of its variation in concentration, composition, hydrogen ion concentration, and buffer action. When it is lacking in buffer action, the results of gluten washing are no doubt greatly influenced by variation in hydrogen ion concentration and buffer action of the flour itself.

The effects on gluten washing of solutions of various concentrations, of different composition and of various pH values have been investigated. Wet and dry gluten and total nitrogen in the dried gluten were determined.

It was found that there was practically no loss in protein when a washed gluten was kneaded under a stream of tap water (containing about 0.025 per cent total solids) for 45 minutes, while with freshly boiled and cooled distilled water there was a loss of over 25 per cent of the protein in that time. A modicum of electrolytes in the wash water is therefore essential in preventing dispersion of gluten proteins during washing.

Sodium phosphate buffer solutions of pH = 7.6 and of concentrations ranging from 0.01 to 1.25 per cent were next compared with tap water and boiled distilled water. A family patent flour was used. There was minimum protein dispersion with buffer solutions of 0.10 and 0.01 per cent concentration and with tap water. The dispersion was somewhat increased with buffer solutions of concentrations of 0.25 per cent and above. It is perhaps significant that buffer solutions ranging from 0.01 per cent to 0.50 per cent gave approximately equal imbibition values. Imbibition was considerably less with the 1.25 per cent buffer solution.

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* Introduced by Carl L. Alsberg.

Finally a series of flours was employed. Some of these were soft wheat flours from which the gluten could be washed only with difficulty. Buffer solutions of pH values 4.4, 5.4, 5.8, 6.8 and 7.6, all of 0.1 per cent concentration were compared with tap water, 0.1 per cent NaCl and 0.1 per cent $CaCl_2$.

With three of the six flours which have been investigated the least protein dispersion occurred with the buffer solution of $pH = 6.8$. In the other three flours there was approximately the same dispersion by buffer solutions of $pH = 5.8$, 6.8 and 7.6 and by tap water. In those flours which were particularly difficult to wash, the least difficulty was experienced with the phosphate buffer solution of $pH = 6.8$.

With each of the six flours, imbibition by the gluten washed out with this buffer solution checked closely with imbibition by the tap water gluten. Imbibition by sodium chloride solution glutens was variable. Imbibition by calcium chloride solution glutens was invariably highest. These calcium chloride glutens were also characteristically incoherent and sticky. This is important in indicating a specific effect of calcium ions on gluten quality. The failure of tap water to produce a similar effect on gluten quality may be due to its lower concentration, to the antagonistic effect of some of its anions, or to its buffer action.

The results suggest that uniform results in gluten washing may be obtained by the use of a sodium phosphate buffer solution which is approximately neutral in reaction and which has a concentration of 0.1 per cent.

269 (2501)

Effects of dehepatization on the reactions of the urinary bladder in canine anaphylactic and histamine shock.

By W. H. MANWARING, V. M. HOSEPIAN, J. R. ENRIGHT
and DOROTHY F. PORTER.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, Calif.*]

In a previous paper[1] it was shown that the urinary bladder is thrown into sharp contraction during the first two minutes of canine anaphylactic shock. Apparently identical reactions of the urinary bladder are produced by the intravenous injection of histamine.

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[1] Manwaring, W. H., Hosepian, V. M., Enright, J. R., and Porter, Dorothy F., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 284.

The bladder contraction in canine anaphylactic and histamine shock is not secondary to the preceding fall in arterial blood pressure. Reduced arterial blood pressure causes the opposite effect, a slight decrease in bladder tone.

The anaphylactic bladder reaction does not take place in dehepatized dogs. With intravenous injections of routine doses of foreign protein the bladder tone remains unchanged throughout the test (five minutes). In contrast with this finding, dehepatization does not abolish the typical bladder reaction to histamine.

We therefore believe that the bladder contraction in canine anaphylactic shock is caused by the sudden formation or liberation of internal hepatic products (hepatic anaphylotoxins), having a histamine-like effect on the urinary bladder. If the blood-free anaphylactic liver is perfused with Locke's solution containing specific foreign protein, the perfusate becomes suddenly opalescent, even milky.

ABSTRACTS OF COMMUNICATIONS.

Minnesota Branch.

Eighteenth Meeting.

Minneapolis, Minnesota, May 7, 1924.

270 (2502)

The nature of the substances adsorbed on the surface of the fat globules in cow's milk.

(Preliminary Report).

By L. S. PALMER and E. SAMUELSON.*

[*From the Section of Dairy Chemistry, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

It is possible to account for the emulsion character of the fat in milk and cream both on theoretical grounds, because of the abundant presence of hydrophilic colloids in milk plasma, and on experimental grounds, because milk plasma (skim milk) readily stabilizes emulsions of oils and fats foreign to milk. However, a number of investigators, beginning with Danilewski and Radenhausen,[1] have attempted to isolate the emulsifying substance from milk. Storch[2] concluded, as the result of extensive experiments, that a special substance, a mucin-like protein, is involved. Völtz and Abderhalden[3] and Völtz[4] decided against the existence of a special substance because of the wide variety of products of varying proportions isolated by them. More recently Bredenberg,[5] who, like Völtz, gives a very complete review of the literature, concluded that a mixture of protein, mucous and fatty substances, including calcium soaps, constitutes the so-called membrane around the fat globules.

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* Alice and Knut Wallenberg Fellow, Royal Agricultural Academy, Stockholm.

By using for the most part physical methods of isolation and purification, except for the fractionation of the final product by solvents, we have secured the following results. When cream was repeatedly diluted with distilled water and recovered by centrifugal separation until the washings no longer give a Fehling's and biuret test, the cream was still a highly stable emulsion. This cream, however, readily formed butter on churning, the buttermilk from which had the appearance of dilute skim milk. A similar fluid was obtained by gently washing the melted butter with warm water until the melted fat rose as a clear liquid.

We have assumed that both the buttermilk and butter washings contained the substances which adhered most closely to the surface of the fate globules in the original cream. Minute fat globules were also present, which were not removed readily, even by the supercentrifuge. In addition, the raw material in the buttermilk had a high ash content, chiefly calcium and phosphorus. The calcium was largely removed by dialysis against distilled water and entirely so by dialysis against water at pH = 4.6, the phosphorus being reduced at the same time to 0.97 per cent of the dry substance.

On the assumption that fat and inorganic salts are the chief

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[1] Danielewski, A., and Radenhausen, P., *Forschung a.d. Gebiet der Viehhaltung, H.* 9, Bremen, 1880.

[2] Storch, E., 36 *Beretning fra dan Kgl. Vet.-og Landbohöjskoles Lab for landökonomiske Försög,* 1897.

[3] Völtz, W., *Pfluger's Arch,* 1904, cii, 373.

[4] Abderhalden, E., and Völtz, W., *Z. physiol. Chem.,* 1909, lix, 59.

[5] Bredenberg, G. A., *Abhandl. Agr. Wiss. Gesell. Finland,* No. 4 1912, 1.

"impurities" present, we found that the emulsion-stabilizing substances adhering to the fat globules in cow's milk apparently consist of a single globulin-like protein free from phosphorus[6] and a mixture of phosphatides of as yet undetermined nature. No glycoprotein was found, the residual traces of sugar in the protein fraction being removed by the solvents which free it from phosphorus. The phosphatides comprised by far the greater part of the total raw material in our experiments. The yield of phosphorus-free protein, which still contained several per cent of other impurities, never exceeded 15 per cent of the total raw material in the buttermilk and butter washings.

The mixture of substances, including the fat globules which resisted mechanical separation, had a gold number of 10 both before and after freeing from calcium by dialysis. In each case this value was changed to 0.5 by boiling the gold sol after the addition of the colloidal mixture and before the addition of the electrolyte. The results make it unlikely that the inorganic impurities in the buttermilk played any part in stabilizing the original cream emulsion.

The occurrence of both hydrophilic and hydrophobic colloids on the surface of the fat globules seems to offer an explanation both for the stability of the cream emulsion and for the relative ease with which the oil-in-water type of emulsion is inverted to a stable water-in-oil type of emulsion which occurs in churning cream to butter.

The detailed report of these experiments will appear later.

271 (2503)

Studies in tuberculosis immunity. I. Diagnostic and sensitizing properties of some new derivatives of tuberculin.

By FREDERICK EBERSON.

[*From the Department of Pharmacology, University of Minnesota, Minneapolis, Minn.*]

This study, preliminary to a series of investigations in tuberculosis immunology, represents an attempt to prepare from tuberculin an active principle which may be of value in the early diagnosis of tuberculosis, particularly in connection with a wider range of specificity for the bovine type of tubercle bacillus; secondly, it is concerned with the sensitizing properties of these derivatives.

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[6] The freeing of the protein from its phosphorus impurities was attended with great difficulty.

Up to the present time no substance has been isolated or prepared from tuberculin such that it will sensitize the animal body to subsequent injections of the the same material, or to its native unaltered tuberculin. The requisite for atypical skin reaction with tuberculin is an infection with tuberculosis. Believing that the specific substance which produces a local tuberculin susceptibility is present in the tubercle bacillus and in tuberculin and is the same element in either case, I have investigated certain derivatives with a view to identify a possible antigenic "nucleus". The failure of tuberculin to develop susceptibility in the sense of a true antibody phenomenon may well be due to technical factors and to conditions related to absorption and dissemination of the material in question. Pharmacologic principles have given abundant evidence of the important rôle played by solubility, diffusibility and penetration of substances used in local and general therapy. These fundamental principles are the same for any biologic reaction between tissues and the substances injected, be these drugs, tuberculins, or proteins in general.

Landsteiner and his collaborators demonstrated some years ago the relation between serologic specificity and chemical structure of antigens. The point of departure in the present series of experiments centered on the properties of modified antigens and the possibility of extending the range of specificity through certain chemical alterations of the protein or non-protein substances in tuberculin.

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The preparation of the derivatives will be described in a complete report at a later date. These derivatives are (1) an acetyl, (2) a benzoyl and (3) an alcohol-soluble, ether-precipitable substance which I have termed "Ether-insoluble X", all of which were derived from human Old Tuberculin. I am indebted to Professor Arthur D. Hirschfelder for suggestions and advice in the preparation of these materials.

Potency of the derivatives was determined by comparing with those of untreated tuberculin the reactions which were obtained by intracutaneous injections at a stated time after inoculation of guinea pigs with human and bovine strains of tubercle bacilli. Weighed amounts of organisms were injected in definite volumes of physiologic salt solution and the skin tests were made by injecting into the skin 0.01 to 0.02 cc. of the derivatives. In several series of animals, totalling 28, which were inoculated with both types of tubercle bacilli, and tested after 11 and 18 days, results were uniformly positive and proved that the derivatives were fully potent and unimpaired by the treatment in the course of their preparation. Uninoculated control animals gave uniformly negative results. The skin manifestations were definite for both the human and bovine types of infection and persisted for a week or more, terminating with deep-seated tissue changes, residual scarring and pigmentation. A small series of 6 tuberculous patients confirmed the results of the animal experiments. In all instances the derivatives gave positive results whenever the Old Tuberculin reacted, with the added important difference that although the tuberculin elicited no response in the animals which were inoculated with bovine tuberculosis, the derivatives produced striking reactions in such heterologous infections.

Experiments were devised to study the diagnostic range of tuberculin derivatives with the following results in a series of 22 guinea pigs: (a) Positive skin reactions were obtained with all derivatives regardless of the type of infection present. (b) Reactions could be elicited as early as 3 days after experimental infection with bovine or human tubercle bacilli, and appeared to vary in intensity with the stage of infection, since most pronounced reactions were obtained 7 or more days after initial infection. In these very early stages tuberculin failed to produce a local response, yet the derivatives, without exception, gave marked reactions. (c) "Ether-insoluble X" gave the best reactions and proved to be a very potent preparation with diagnostic possibilities. A series of 6 guinea pigs which were tested by intracutaneous injections after 4, 8, 15, 29 and 152 days gave pronounced reactions for human and bovine infections. The local response could be elicited repeatedly in the course of the disease. Control animals were uniformly negative. (d) Typical reactions which followed intracutaneous injections of acetyl and benzoyl derivatives did not interfere with the development of an intense local reaction to "Ether-insoluble X". This observation is significant because the reverse was found to be the case in animals which were uninoculated and treated with the same derivatives prior to intracutaneous tests with the "Ether-insoluble X".

Sensitizing properties of tuberculin derivatives were studied in several series of guinea pigs which were divided into groups of healthy and infected animals. In the former group, numbering 8, the animals were given repeated subcutaneous injections of the different derivatives, using one preparation at a time, and then tested intracutaneously with each of the homologous and heterologous derivatives and also with untreated Old Tuberculin. Intense reactions developed in these animals after 24 hours, reached a maximum after 48 to 72 hours and persisted for 5 to 21 days. The lesions were of variable extent but were typical of true skin reactions. Four control animals which were tested with one each of the derivatives and Old Tuberculin, gave negative results. The "Ether-insoluble X", injected intracutaneously 55 to 66 days after an initial injection of the other two derivatives, failed to elicit a local response in three guinea pigs which were so tested in this series. An explanation for this interesting observation may be found in the fact that these animals had by this time received numerous injections of the derivatives, with the result that a state of resistance or immunity had been developed from an initial state of hypersusceptibility. A striking demonstration of sensitization was observed in another group of 5 animals which showed marked symptoms of protein intoxication after subcutaneous injections of acetylated derivatives in the course of systematic treatment over a period of several weeks. Within a few minutes after the injection, dyspnea and weakness were noted and the animals became stuporous. Recovery took place within 12 hours. A loss of weight occurred regularly in these animals, until a tolerance to the substance had been developed. Infected guinea pigs presented a somewhat different picture. In a series of ten animals with bovine and human tubercle infection, acetylated derivative was injected subcutaneously in one cc. doses at intervals of one week. The first dose was given 11 to 21 days after inoculation. In the course of this treatment it was observed that the animals developed a diffuse reddening over the entire abdomen and showed in addition a flare-up of old intracutaneous sites of injection. In some cases where cold abscesses were present, an intense reddening of these areas was observed. With each subsequent injection of the derivative, the animals showed similar local reactions. After the fourth and last injection in this series, all of the animals developed local areas of infiltration and edema varying in size from 1.0 x 2.0 cm. to 3.0 x 3.5 cm., with central purplish zone of about 1.0 to 1.5 cm. in diameter. These reactions persisted for several days and subsided with residual pigmentation. One of the guinea pigs manifested a typical anaphylactic reaction with dyspnea weakness, involuntary micturition and defacation, and distress lasting 30 minutes, during which time it lay on its side. These final injections were made 36 to 44 days after initial inoculation with tuberculosis of human and bovine origin. The series of five guinea pigs with bovine infection gave results somewhat different. In general, the reactions were not so intense and the size of the lesion was rarely over 1.0 cm. in extent. In one animal local necrosis developed with some erythematous changes in the remainder local infiltration and edema were observed.

These derivatives are being studied in a large series of tuberculous patients, adults and children. There is evidence at hand to show that sensitization can be demonstrated even in terminal cases. Furthermore, typical skin reactions can be elicited in children who have failed to react to human tuberculin while reacting to bovine, and vice versa.

272 (2504)

Observations on the mechanism of the tyrosine-tyrosinase reaction.

By ROSS AIKEN GORTNER.

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Raper and Wormall[1] in a recent paper conclude as follows:

"Tyrosinase first produces from tyrosine a red substance. This process requires the presence of the enzyme and only takes place in the presence of oxygen. This red substance becomes colorless spontaneously and is then oxidized to form melanin. These last two processes take place in the absence of tyrosinase, but may be accelerated by it or by other oxidases found in potato juice."

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[1] Raper, H. S., and Wormall, A., The tyrosine-tyrosinase reaction, *Biochem. J.*, 1923, xvii, 454.

While this statement may be correct in so far as potato tyrosinase is concerned, it is apparently incorrect as a generalization for all tyrosinase preparations. I have shown[2] that the meal worm, *Tenebrio molitor,* contains a very active tyrosinase which can be prepared in an insoluble form. When this insoluble tyrosinase is used, it is possible to remove the enzyme completely from a system by the simple process of filtration. Consequently it is possible to initiate a reaction, carry it to a definite stage, and then stop the action of the enzyme by filtering the reaction mixture.

Tyrosine and the insoluble tyrosinase were allowed to react until a deep rose color had developed. One-half of the mixture was now removed and filtered twice through "barium" filter papers to remove the enzyme. The other half of the mixture served as a check. Both tubes were set aside in the dark, but open to the oxygen of the air. In a few hours, the contents of the tube containing the unsoluble tyrosinase and tyrosine solution became changed, first becoming violet color and later depositing the black pigment in the usual manner. The filtered portion, on the other hand, remained an unchanged rose color for eighteen days and was then discarded.

Certain evidence has already been presented[3] which indicates that the initial red or rose color is dependent upon the presence of phenolic group in tyrosine while the amino group is necessary for the production of the black pigment. Tyrosine [β (*p*-hydroxy phenyl) α amino propionic acid] was changed to tyrosol [β (*p*-hydroxy phenyl) ethyl alcohol] by yeast fermentation according to the method of Ehrlich.[4] The tyrosol treated with soluble tyrosinase from potatoes or mushrooms *(Agaricus campestris)* or with the "insoluble" tyrosinase from meal worms was oxidized with the development of a rose red color, *which remained in solution* and which was not altered on long standing. No insoluble pigment precipitated. The onset of the rose color occurred in solutions of tyrosine and tyrosol after approximately the same time interval following the addition of the enzyme so that apparently the same reaction occurs in both compounds. The lack of black pigment formation in the case of the tyrosol experiments is apparently due to the absence of the amino group which would, at least, suggest that the rose-red oxidation colors of a tyrosine-tyrosinase system are dependent upon one type of a chemical reaction and the red-black oxidation colors are caused by a second reaction. It is my experience that the enzyme is necessary to initiate each reaction, and that the second phase of the oxidation will not take place spontaneously, even in the presence of atmospheric oxygen.

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[2] Gortner, R. A., A contribution to the study of oxidases, *Trans. Chem. Soc.*, (London) 1910, xcvii, 110.

[3] Gortner, R. A., Studies on melanin III. The inhibitory action of certain phenolic substances upon tyrosinase. A suggestion as to the cause of dominant and recessive whites. *J. Biol. Chem.*, 1911, x, 113.

[4] Ehrlich, F., *Ber.*, 1911, xliv, 139.

273 (2505)

The distribution of water added to blood between the corpuscles and the serum.

By G. L. KING and F. H. SCOTT.

[*From the Physiological Laboratory, University of Minnesota, Minneapolis, Minn.*]

There seems to be uniformity among those who have tried that distilled water injected intravenously or subcutaneously does not act as a diuretic. While the water content of the whole blood or the hemoglobin percentage has been followed the actual water content of the plasma does not seem to have been determined. Owing to the large percentage of solid in red corpuscles it seemed possible that these cells might take up considerable water if the conditions of equilibria were upset. In this paper reference will be made only to experiments done *in vitro*. Two methods were followed. In the first, various amounts of water were added to samples of the same defibrinated blood, and after standing, the various samples were centrifuged and the water estimated in the different sera by weighing. In the second method the defibrinated blood was centrifuged and the serum and corpuscles separated. Portions of the serum were diluted with definite amounts of distilled water and then equal volumes of the centrifuged corpuscles and various sera were mixed and allowed to stand for a couple of hours. The results show that if the amount of water added to the blood is not sufficient to cause perceptible laking, far more of the water enters the corpuscles than would be expected from purely osmotic considerations. In some of our experiments all of the water added to the blood had entered the corpuscles. An example of this is given below.

Ox defibrinated blood. Original serum had 91.5 per cent water. Dilutions made by taking 14, 13, 12 and 11 cc. of this serum and adding water to make up to 15 cc. 10 cc. of corpuscles mixed with 10 cc. of the sera. After standing 2½ hours and centrifuging, the percentages of water in the sera were: 90.9, 91.2, 91.5 and 91.2 respectively. By the hematokrit the percentage of corpuscles was 44.1 in the original, 48.4, 49.4, 53.5 and 56.4 in the bloods with diluted serum.

The average water content of the serum increased only from 90.9 to 91.5 in eight experiments with different samples of ox blood when 25 cc. of water was added to 100 cc. of the blood. If one takes the original blood as 44 per cent corpuscles and 56 per cent serum and the added water distribution uniformly between the two, one would expect about 92.7 per cent water in the serum instead of the 91.5 per cent found. These experiments were carried out at room temperature.

These results show that water may penetrate the cells beyond the amount expected from a purely osmotic process and must be due to some hydrophilic property of the colloid. These experiments are being continued, using different strengths of salt solution.

274 (2506)

Production of goiter in rats by restricted iodin feeding.

By EDW. M. HAYDEN, W. T. WENNER and C. W. RUCKER.*

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

Two litters of white rats were divided, half of each being placed in a separate cage. They were all fed the following diet: 53 parts oats, 25 parts patent flour, 20 parts linseed meal, one part calcium phosphate and one part sodium chloride. In one cage the rats were given distilled water to drink, and the other, water containing 0.1 mg. of iodine per liter. Two rats on each diet were killed after 4 weeks and the weighing of the thyroid completed within 7 minutes after death. This process was repeated every 2 weeks until all had been killed. The following table shows the thyroids to be doubled in size in rats not receiving iodine:

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* Introduced by E. P. Lyon.

	Iodine-rich.			Iodine-poor.	
Rat	Body wt.	Thyroid	Rat	Body wt.	Thyroid
1.	90 gm.	9.9 mg.	1.	90 gm.	15.7 mg.
2.	99 gm.	10.0 mg.	2.	97 gm.	23.3 mg.
3.	114 gm.	9.9 mg.	3.	127 gm.	33.3 mg.
4.	135 gm.	14.2 mg.	4.	133 gm.	27.6 mg.
5.	137 gm.	12.6 mg.	5.	137 gm.	25.5 mg.
6.	140 gm.	15.3 mg.	6.	140 gm.	30.1 mg.

275 (2507)

The reaction of tubercular serums to phenols.

By I. A. MONTANK.*

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

In continuing the investigations on the precipitin reaction in tuberculosis begun by Larson, Montank and Nelson,[1] it was found that certain non-specific substances would serve as "antigens".

If a serum from a tuberculous patient is covered with dilute solution of thymol, toluene, phenol or tricresol a cloud is formed at the interface of the two fluids. The reaction occurred with such regularity in the tests on tuberculous serum that it was considered worthy of further investigation. Tricresol was selected as the reagent to be used and made up to a concentration of 0.2 of one per cent in physiologic saline solution. The serums to be tested were covered with the reagent and incubated at 37° C. for a period up to 2 hours. The positive reactions often appeared in the first few minutes. In the far advanced cases the reaction developed more slowly, and in some cases, a week or more before death ensued, a negative result was obtained. Normal guinea pig, sheep, and rabbit blood serums gave negative results when tested with the reagent. Serum from guinea pigs with moderately advanced tuberculosis gave positive reactions, while those in the last stages of the disease usually gave negative tests.

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* Introduced by W. P. Larson.

[1] Larson, W. P., Montauk, I. A., and Nelson, E., Proc. Soc. Exp. Biol. and Med., 1923, xx, 350.

Thus far the blood serum of 3030 human cases have been examined. Of these, 2286 were students entering the university. Four hundred and eighty-eight were patients in the University Hospital and Dispensary, but not in the tubercular clinic. Two hundred and fifty-six cases, representing all stages of tuberculosis, were from local sanatoriums. From the 2,286 students seven positive reactions were obtained. Two of these have since been found to have tuberculosis, although at the time of the test there were no other symptoms of tuberculosis to be found. Of the 488 cases not suspected of having tuberculosis, 61 gave positive reactions. Eleven of this group were found to be definitely tuberculous. In 22 cases the symptoms recorded were of such a nature as to be suggestive of tuberculosis. In the remaining 28 cases the charts revealed nothing suggestive of tuberculosis. Of the 256 known tubercular sanatorium cases, 238 were positive and 18 negative. Eleven of the negative cases were either cured or arrested. In the 7 remaining cases, 1 was far advanced, 4 were cases of bone, and 2 of pulmonary tuberculosis.

Rabbits immunized against typhoid bacilli, staphlococcus, human blood serum and a polyvalent vaccine containing the organism of *Streptococcus, Staphlococcus, Pneumococcus, Micrococcus tetragenes* and the *Pneumobacillus* all gave negative reactions with the reagent, although in each of the immune serums a high value of specific antibody content was demonstrated by the use of the agglutination, precipitin and complement fixation tests. Anti-pneumococcus horse serums also gave negative reactions with the reagent. On the other hand, the serum of a rabbit immunized with tubercle bacilli gave a very heavy ring when treated with the reagent.

It was also found that serums from apparently normal persons suffering from acute colds gave positive reactions for a few days when the cold was at its height.

In a few incipient cases of tuberculosis the reaction was not as pronounced as in the moderately advanced cases, nevertheless, it was usually very definite. In one instance, the serum from a definitely exposed case gave a positive reaction with the tricresol reagent two months in advance of any tuberculous symptoms or positive sputum findings.

276 (2508)

The relation between body-length and body-weight in the human embryo and fetus.

By RICHARD E. SCAMMON and LEROY A. CALKINS.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.*]

It has been generally assumed that the weight of the body in prenatal life may be represented by a cube of a fraction of the total or crown-heel length. In a study of the lengths and dead-weights of 2202 embryos and fetuses we have found that this relation can be more accurately expressed by the formulae:

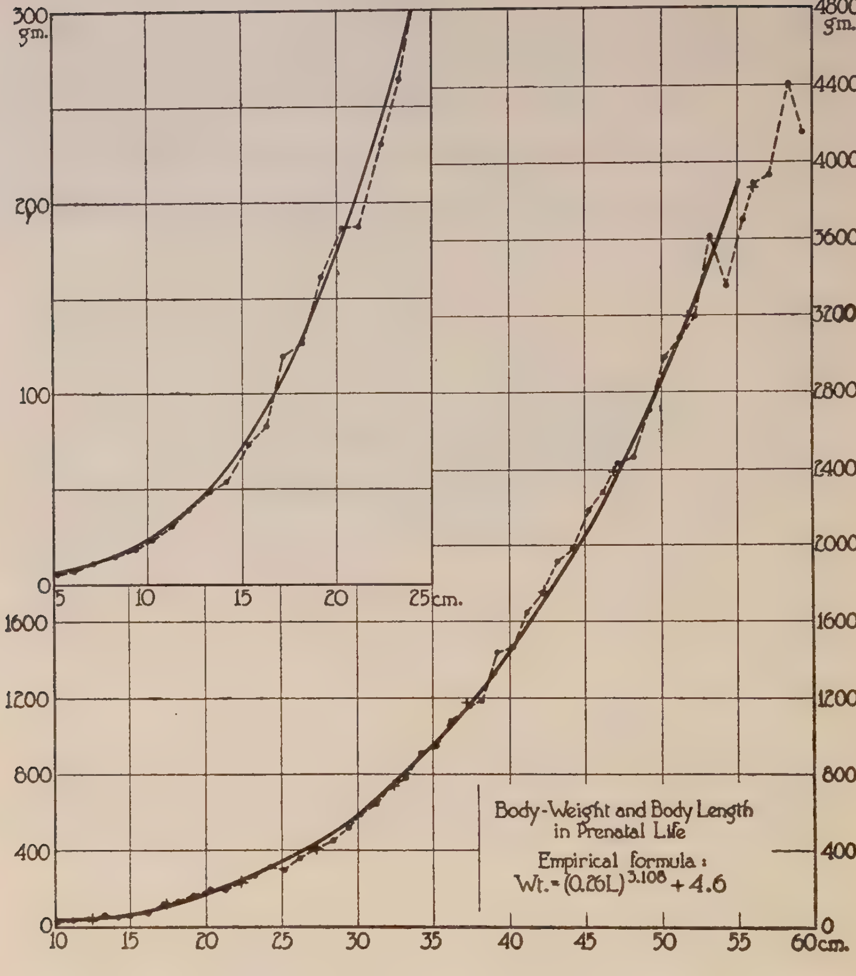
$$(1)\quad W = (0.26\,L)^{3.108} + 4.6, \text{ or}$$

$$(2)\quad L = 3.846\,\sqrt[3.108]{W - 4.6}$$

In these expressions W is the weight in grams, and L is the total or crown-heel length in centimeters. These formulae are satisfactory from 5 to 55 cm. body-length but do not hold good above the latter limit and, probably, not below the former. The following graph shows the relation between the observed values and the calculated curve of weight increase, in respect to length; and the accompanying table gives the observed and calculated mean weights for each 5 cm. range of body-length, and the absolute and percentage differences between these values.

The live and dead-weights of fetuses of the same body-length are quite different, the former being distinctly the greater. Therefore the majority of the published tables and curves of weight increment in prenatal life are not strictly correct, for they include both types of material.

In order to estimate the amount of this difference we have computed the mean body-weight of living fetuses and newborn children for each 5 cm. range of body-length from 35 to 55 cm. inclusive. These computations are based on a series of 5674 observations. A comparison of these values with the calculated values for dead material at the same mean lengths shows an average difference of 219.3 gm. in favor of the living material. The 5 cm. range differences showed no consistent increase with increasing body-length. Therefore the relation between live-



Body-Weight and Body Length in Prenatal Life

Empirical formula: $\text{Wt.} = (0.26L)^{3.108} + 4.6$

weight and body-length in the latter part of prenatal life may be approximated by the empirical formulae:

$$(3)\quad W = (0.26\ L)^{3.108} + 223.9, \text{ or}$$

$$(4)\quad L = 3.846\ \sqrt[3.108]{W - 223.9}$$

No consistent sex differences in the relation of body-weight to body-length were noted in either the living or the dead material.

Calculated and Observed Dead-weight and Length in Prenatal Life.
Empirical formula:
$\text{Wt. (gm.)} = [0.26 \text{ Lth. (cm.)}]^{3.108} + 4.6.$

Length (cm.)		Weight (gm.)		Deviation of calculated number from observed values		
Range	Mean	Observed	Calculated	Absolute (gm.)	Relative (per cent)	Number of cases
5-10	7.7	13.05	13.08	+0.03	+0.19	33
10-15	12.3	40.54	41.68	+1.14	+2.82	68
15-20	17.3	114.61	111.65	—2.96	—2.58	78
20-25	22.3	238.71	241.24	+2.53	+1.06	117
25-30	27.2	405.08	441.49	+36.41	+8.99	143
30-35	32.3	750.31	749.91	—0.40	—0.05	194
35-40	37.2	1163.16	1160.67	—2.49	—0.21	328
40-45	42.2	1758.39	1715.51	—42.88	—2.44	414
45-50	46.9	2389.17	2380.03	—9.14	—0.38	456
50-55	51.7	3204.89	3220.47	+15.58	+0.49	371
		Sum of deviations		113.56	19.21	2202
		Unweighted mean deviation		11.36	1.92	
		Weighted mean deviation		15.63	1.42	

ABSTRACTS OF COMMUNICATIONS.

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277 (2509)

The purification of jack bean urease.

By JAMES B. SUMNER, VI A. GRAHAM and CHARLES V. NOBACK.

[*From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, N. Y.*]

Thirty per cent alcohol extracts of jack bean meal contain the enzymes urease and amylase associated with the globulins canavalin and concanavalin A and B, albumin, proteose, pentose and hexose carbohydrates and a yellow coloring matter. On cooling the alcoholic extract to —5° C. the urease is almost entirely precipitated and can readily be centrifuged off. This procedure if repeated separates the urease from everything but the three globulins. It is best, however, to treat the precipitated urease with distilled water when the concanavalin B will crystallize out of solution completely. The material is now centrifuged and after the addition of a considerable amount of neutral potassium phosphate solution and alcohol to 30 per cent is chilled and centrifuged a second time. The urease is largely precipitated but not so completely as the first time. The precipitate is dissolved in distilled water, seeded with a few srystals of concanavalin A and allowed to stand for several days in the ice chest. Eventually all of the concanavalin A will crystallize out. After centrifuging neutral phosphates are added and alcohol to 30 per cent. The material is allowed to stand for several days in the ice chest. The urease slowly becomes insoluble. This reaction is accompanied by a diminution in activity which we believe is due merely to the decrease in dispersion. The insoluble urease can be centrifuged off and washed many times with phosphates and with distilled water. Prepared in this manner urease is almost completely insoluble in salt solution and contains about 16 per cent of nitrogen. It gives all of the protein color reactions and has an activity of 15000 units per gram of protein. The urease is probably not in a state of adsorption for it cannot be extracted by neutral phosphate solution. The material is not composed of canavalin for canavalin is not denaturized on standing in dilute alcohol. Strong trypsin solutions dissolve insoluble urease at room temperature and in doing so the activity of the urease is a little more than doubled. This effect may be due to the increased dispersion. Urease has been prepared several times by adsorption methods with an activity of about 30000 units per gram of protein present. This seems to be the limit of the process of purification.

Insoluble urease, weight for weight, is 140 times more active than the original meal and is a comparatively stable substance. To the best of our present knowledge it is an individual protein.

278 (2510)

The energy metabolism of normal, full term infants.

By RUTH CONKLIN, M. ELIZABETH MARSH and JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester and the Obstetrical Division of the Highland Hospital, Rochester, N. Y.*]

Upon 50 infants under 2 weeks of age, 234 observations of respiratory metabolism were made but of this number only 108 were truly basal. The methods used were all exactly as described previously for the study of premature infants.[1]

The respiratory quotient and also heat production varied with age. The R. Q. was lowest on the second day and gradually rose. Heat production per square meter of body surface (Lissauer formula) was lowest on the 4th day, remained practically constant until the 8th day, and then rose.

Total heat production was found to be 6.67 calories per hour when all basal periods were averaged. Heat expressed per kilo of body weight per hour was 1.86 calories where only minimal production for each child was averaged but for all basal periods was 2.00 calories with a coefficient of variability of 13.0 per cent. When expressed per square meter per hour the average for minimal was 27.42 calories and for basal 29.16 calories with a coefficient of variability of 11.9 per cent.

Assuming that the Lissauer formula, $10.3\sqrt[3]{W^2}$, gives an essentially correct surface area for the infant, surface area is a slightly better measure of metabolism than weight, since the coefficient of variability is slightly lower.

As a measure of metabolism, pulse must be considered a very poor criterion since the coefficient of correlation between the two was found to be 0.178, whereas a perfect correlation is represented by unity.

Increases in heat production due to muscular activity went as high as 56 per cent in successive periods and as high as 117 per cent when maximum was compared with minimum for the whole series of observations on a single infant.

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[1] Marsh, M. E., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 523.

In several cases a material increase in metabolism was obtained when the amount of the feeding was increased over the amount given before a preceding observation. Feedings of 10 per cent lactose and also dextrose solutions were given in several cases, particularly the first three days after birth and in most instances were as effective in increasing heat production as breast milk.

279 (2511)

Regeneration of hemoglobin and diet factors in prolonged, severe experimental anemia.

By G. H. WHIPPLE and F. S. ROBSCHEIT-ROBBINS.

[*From the Department of Pathology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.*]

Our earlier observations were made upon dogs rendered anemic by two or three large hemorrhages. Following this sharp reduction of blood hemoglobin from about 120 per cent to approximately 60 per cent, we observed the curve of blood hemoglobin regeneration as it returned to a normal level. It was easy to show that under these conditions a maximal hemoglobin regeneration was induced by certain diets—for example, liver and meat feeding. Many other diet factors were almost inert and certain drugs (iron and arsenic) were inert in this type of experiment.

The data given in this paper are derived from experiments on dogs under very different anemic conditions but the methods used are similar to those described in recent papers.[1] The *anemia level* is maintained as near a constant level as possible by frequent bleedings. The number and amount of bleedings are determined by the hemoglobin, hematocrit and plasma volume figures. Under such conditions the animal is lively, active and healthy but with a constant hemoglobin level of 40 to 50 per cent. We assume, therefore, a constant and maximal stimulus to blood hemoglobin production. Such conditions should test the potential hemoglobin factors in any given diet.

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[1] Whipple, G. H., Hooper, C. W., and Robscheit, F. S., *Am. J. Physiol.*, 1920, liii, 2, 151.

A great deal of experimental work was needed to devise a basal bread ration which should be a complete food, palatable to the dogs yet producing the *minimal hemoglobin regeneration*. The bread which we are using at present meets these requirements and dogs can be maintained on this diet alone for months in good health. This bread is made in the laboratory and consists of the following ingredients: Wheat flour (bread) 12,000 gm., potato starch 6,000 gm., wheat bran 2,000 gm., sugar 4,000 gm., salt mixture (McCollum and Simmonds, minus iron citrate) 120 gm., cod liver oil 2,000 cc., canned tomato 2,000 gm., yeast (baker's cake) 454 gm., water 7,500 cc.

A glance at the sample tables (A and B) shows two standard experiments in condensed form. All these meat foods are boiled in water and hashed before feeding. The diet list favorable for hemoglobin production in this type of experiment is headed by liver and kidney. Beef heart muscle is more favorable than beef skeletel muscle. Salmon muscle is practically inert and has a minimal influence on hemoglobin construction in the body. Egg yolk is quite favorable, much more so than egg white which, however, is not inert. Red cells or hemoglobin given by mouth or hypodermically are distinctly favorable for hemoglobin production. It is a bit surprising that brains exert a favorable influence on hemoglobin production. Pancreas and thymus feeding have little if any effect on hemoglobin regeneration. Fresh tripe boiled has a slightly favorable effect on hemoglobin production but a very interesting influence upon the blood plasma which shows a great increase.

Under the conditions of these long continued experiments we note that iron (Blaud pills) has a distinctly favorable influence on hemoglobin production. Arsenic (sodium cacodylate), however, is quite inert. Ox or pig bile is likewise inert and the pigments if absorbed are not used in hemoglobin construction.

It will be noted that the bread feeding often causes no hemoglobin regeneration over and above the *maintenance factor* which is always present and presumably constant. Some dogs may give as much as 5 gm. of hemoglobin surplus a week on the standard bread ration. There are likewise individual variations in reaction to standard diet factors—for example, beef muscle. This question cannot be discussed at this time for lack of space.

Dog No. 19-104.

TABLE A.

Diet periods 1 week each	Food consumption	Weight		Hemoglb. removed bleeding	Blood Hemoglb. 100 %= 13.8 gm.	Plasma vol.	Remarks
		Loss	Gain				
	per cent	lbs.	lbs.	gm.	Per cent	cc.	
Standard bread 350 gm.	28	1.9	—	0	57	576	
Standard bread 350 gm.	56	0.9	—	0	56	730	22.8 lbs.
Liver 400 gm.	100	—	0.9	10.9	86	672	
Liver 400 gm.	100	0.3	—	69.3	66	618	23.4 lbs.
Standard bread 350 gm.	56	0.9	—	27.9	54	640	Liver carry over
Standard bread 350 gm.	69	0.1	—	0	48	632	22.3 lbs.
Whole red cells 10 gm. + bread 350 gm.	95	—	1.7	0	61	689	
Whole red cells 10 gm. + bread 350 gm.	93	—	0.5	13.9	43	753	
Whole red cells 10 gm. + bread 350 gm.	100	—	0.2	0	47	772	
Whole red cells 10 gm. + bread 350 gm.	100	—	1.0	12.9	43	706	25.6 lbs.
Hemoglb. intrap. 12 gm. + bread 350 gm.	98	—	0.4	0	51	694	Daily perit. inj. ave. 2 gm.
Hemoglb. intrap. 34 gm. + bread 350 gm.	68	0.4	—	29.0	44	752	Daily perit. inj. ave. 5 gm.
Standard bread 350 gm.	66	0.8	—	15.1	42	718	Slight carry over
Standard bread 350 gm.	61	0.5	—	0	46	650	24.3 lbs.
Standard bread 350 gm.	60	1.0	—	0	48	662	
Standard bread 350 gm.	45	0.9	—	13.2	42	616	24.3 lbs.
Canned salmon 200 gm. + bread 300 gm.	94	—	2.6	0	42	744	
Canned salmon 200 gm. + bread 300 gm.	85	—	0.1	0	38	732	25.0
Canned salmon 200 gm. + bread 300 gm.	38	0.4	—	0	36	814	Increased plasma volume
Beef heart 300 gm. + bread 200 gm.	88	—	2.4	0	49	723	
Beef heart 300 gm. + bread 200 gm.	98	—	0.4	17.9	49	776	27.4 lbs.
Standard bread 350 gm.	22	3.9	—	0	53	638	23.6 lbs.
Egg albumin 150 gm. + bread 250 gm.	29	1.6	—	11.5	38	625	22.0 lbs.
Liver 100 gm. + bread 250 gm.	100	—	2.3	0	52	606	
Liver 100 gm. + bread 250 gm.	100	0.1	—	28.5	53	646	24.3 lbs.
Standard bread 350 gm.	81	1.3	—	15.2	37	606	
Standard bread 350 gm.	14	2.0	—	0	36	544	21.0 lbs.

Dog No. 21-64

TABLE B.

Diet periods 1 week each	Food consumption	Weight		Hemoglb. removed bleeding	Blood Hemoglb. 100 % = 13.8 gm.	Plasma vol.	Remarks
		Loss	Gain				
	per cent	lbs.	lbs.	gm.	per cent	cc.	
Standard bread 450 gm.	46	2	—	14.0	40	818	
Standard bread 450 gm.	64	—	0.9	0	36	966	31.9 lbs.
Liver 450 gm.	100	0	0	0	80	866	
Liver 450 gm.	100	1	—	47.3	97	747	30.8 lbs.
Standard bread 450 gm.	67	—	0.9	26.6	59	906	
Standard bread 450 gm.	100	—	1.0	0	55	894	32.7 lbs.
3 Blaud's pills + bread 450 gm.	89	0.4	—	0	80	826	
3 Blaud's pills + bread 450 gm.	82	0.6	—	43.6	47	960	31.6 lbs.
Standard bread 450 gm.	85	0.5	—	0	63	959	31.1
Standard bread 450 gm.	49	1.3	—	18.3	44	934	Bleeding 1st day of 2nd wk.
Chloroform orally + bread 450 gm.	44	2.1	—	0	48	813	Chloroform 2 dos. 2 cc. ea.
Standard bread 450 gm.	55	—	0.1	0	43	816	27.8 lbs.
Liver 400 gm. + bread 300 gm.	96	—	4.6	20.6	46	952	
Liver 400 gm. + bread 300 gm.	100	—	1.7	28.3	45	1000	34.1 lbs.
Standard bread 450 gm.	90	1.0	—	0	57	956	
Standard bread 450 gm.	100	0.2	—	30.9	43	1000	32.9 lbs.
Sodium cacodylate sub. + bread 450 gm.	87	—	0.1	0	45	1017	1 cc. of 1 per cent sol. daily
Sodium cacodylate sub. + bread 450 gm.	88	0.6	—	0	46	967	32.4 lbs.
Beef kidney 400 gm. + bread 300 gm.	100	—	4.1	26.0	51	972	
Beef kidney 400 gm. + bread 300 gm.	100	—	1.4	35.1	56	849	37.9 lbs.
Standard bread 450 gm.	69	2.6	—	17.5	60	892	
Standard bread 450 gm.	87	—	0.5	15.5	58	866	35.8 lbs.
Egg yolk 85 gm. + bread 350 gm.	100	—	0.1	19.0	69	850	
Egg yolk 85 gm. + bread 350 gm.	100	—	0.3	44.4	47	818	35.9 lbs.
Standard bread 450 gm.	100	—	0.8	23.1	56	902	
Standard bread 450 gm.	72	1.8	—	15.5	40	888	35.2 lbs.
Egg albumin 150 gm. + bread 350 gm.	100	—	1.0	0	45	980	36.2 lbs.

Following a very favorable diet (for example, liver) we note a *storage* of potential material which, in the next week or two of unfavorable diet, will be responsible for the production of a considerable excess of hemoglobin. We may speak of this as a carry over due to storage of parent substances capable of manufacture into hemoglobin. It is probable from our feeding experiments that this substance is stored in the liver and kidney in considerable quantity. On a normal diet dogs always store in the body a considerable reserve of material which can be built into hemoglobin when the diet is changed to one unfavorable to hemoglobin production.

The importance of plasma volume determinations is evident from an examination of the tables and often an apparent increase in hemoglobin value is explained by the blood plasma volume decrease rather than by actual hemoglobin increase. Change in plasma volume with change of body weight due to favorable diet is illustrated. The complete experimental data will be published in the near future.

280 (2512)

The effect of potassium on the metabolism of surviving muscle tissue.

By FRED R. GRIFFITH, JR.*

[*From the Laboratories of Physiology of the University of Buffalo, Buffalo, N. Y., and the Harvard Medical School, Boston, Mass.*]

The following experiments were undertaken in order to determine whether the pronounced effect of potassium on the contractile process in skeletal and cardiac muscle is associated with changes of metabolism, as indicated by variations in the rate of acid production.

Isolated frog tissues were used in all the experiments and their "normal" rate of acid production was determined in Ringer's solution before treating them with potassium. The Ringer's solution consisted of a mixture of the three chlorides, in the usual concentration for cold-blooded tissues, and the potassium effect was obtained by immersing the muscle in an isotonic solution of potassium chloride. Precaution was taken to have the Ringer's and potassium chloride solutions of the same pH and buffer capacity. The experiments were carried out at various room temperatures, but never did the temperature vary significantly during any one experiment. The experiments fall into three classes, as follows:

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* Introduced by Wayne J. Atwell.

1. *The effect of potassium on the total acid production of skeletal muscle.* Sartorius muscles were used in these experiments and their rate of total acid production was measured by the indicator method of Haas,[1] using phenol red as indicator. Determinations were made of the time, in seconds, required by a muscle to produce sufficient acid to cause an increase in the acidity of the solution it was in from pH 7.381 to pH 7.168. Each determination was made with the muscle immersed in 2.5 cc. of solution (Ringer or KCl) in a hard-glass test tube of 3 cc. capacity, stoppered with a paraffined cork. At least two determinations were made of the rate of acid production in Ringer solution, in order to establish the "normal", before treating the muscle with potassium; it was then immersed, without any delay, in isotonic potassium chloride solution, previously adjusted to the same pH and buffer capacity as the Ringer.

Twenty-six experiments of this kind were performed and they all concurred in showing that the rate of acid production is increased under the influence of potassium. The average increase for the entire series was approximately 200 per cent. This increase occurs immediately, reaching its maximum within 5 to 10 minutes and then subsides until the rate is again "normal" within 40 to 60 minutes.

2. *The effect of potassium on the carbon dioxide production of skeletal muscle.* These experiments were undertaken in order to determine to what extent carbon dioxide might be responsible for the increased acid production described in the preceding section. Carbon dioxide production was measured by a modification of the Osterhout technique, which has been previously described.[2] In order that the carbon dioxide production would be

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[1] Haas, *Science*, N. S., 1916, xliv, 105.

[2] Griffith, *Am. J. Physiol.*, 1923, lxv, 15.

great enough for rapid determination by means of this method, both hind legs of a frog were used in each experiment, rather than a single muscle. This might be expected to lead to differences in regard to the relative magnitude and time relations of the effect in these, as compared with the preceding experiments on account of the slower rate of penetration of the potassium ion into large, as compared with small, (sartorius) muscles. With the exception of slight differences in magnitude and duration which probably have their origin in this difference in method, the results of this series of experiments (12 experiments in all) show a fair correspondence with those just described. Thus, the carbon dioxide production was increased in rate about 150 per cent; the maximum was attained within 20 to 30 minutes; and the rate had not returned to "normal" until the end of 1½ to 2 hours.

These experiments make it probable, therefore, that the increased acid production, described in the preceding section, consists in large part, if not entirely, of carbon dioxide.

3. *The effect of potassium on the total acid production of cardiac muscle.* The marked difference in the effect of potassium on the contractile process in skeletal and cardiac muscle makes a comparison of its effect on their metabolism of interest.

Frog hearts were used in these experiments and their rate of acid production was determined by the method described in Section I. In many of the experiments the entire heart, including the sinus, was used and such hearts were beating regularly until they were treated with potassium. This might seem to invalidate the comparison of the effect of potassium on this tissue with its effect on quiescent skeletal muscle. In other experiments, therefore, the sinus was cut off at the time of removal of the heart from the body and these hearts were observed not to have resumed beating during the preliminary determinations of their "normal" rate of acid production in Ringer. This factor appeared not to influence the results, for in all cases the rate of acid production progressively diminished after immersion in the potassium chloride solution. This conclusion is based on the results of 18 experiments with hearts that were quiescent or were beating at rates varying from 24 to 64 per minute and in which the potassium effect was produced at times varying from 20 minutes to 7 hours during the survival period.

In order to evaluate these results it became necessary to establish the "normal" course of survival acid production for heart muscle during such periods as were used in these experiments. This was found to diminish in a manner similar to that found by Fletcher[3] for the survival carbon dioxide production of skeletal muscle. And, by comparison with this "normal" decrease, it became apparent that potassium causes an immediate and pronounced drop in the rate of acid production to a magnitude approximately 75 per cent below what might have been expected if the tissue had remained in Ringer.

281 (2513)

Changes in the blood sugar and blood gases during exercise.

By FRANK A. HARTMAN and FRED R. GRIFFITH, JR.

[*From the Department of Physiology, University of Buffalo, Buffalo, N. Y.*]

It has been found by Scott and Hastings[1] that exercise causes a slight decrease in blood sugar and an increase in the oxygen content of the blood of dogs when they are made to work on an electrically driven horizontal treadmill at the rate of five miles an hour. We have made a study of the influence of exercise on the blood sugar and blood gases of several cats and one dog. The animals were exercised in a circular treadmill with a tread 5.75 meters long. Blood was collected under paraffin oil from an incision in an ear vein. The gases were analyzed immediately by Van Slyke's method of determining[2] the oxygen and carbon dioxide in one cubic centimeter of blood. Sugar was determined by the method of Hagedorn and Jensen.[3]

Ten cats were studied in which the blood sugar alone was determined. With five other cats and the dog the sugar, oxygen and carbon dioxide of the blood were determined. In the latter set, two or three experiments were performed with each animal. In all but one case the cats were exercised by driving the treadmill by hand. The animals usually worked from ten to thirty minutes travelling from 500 to 1500 meters. In every instance where this was done, the blood sugar invariably increased more than 100 per cent, occasionally as much as 400 per cent. In these experiments the carbon dioxide was lowered markedly, commonly to twelve volumes per cent and occasionally as low as eight volumes per cent. This was apparently caused by the very rapid breathing accompanying the strenuous exercise. The oxygen was at first increased and then later decreased in some experiments. In other experiments the oxygen was decreased without a previous period of increase.

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[3] Fletcher, *J. Physiol.*, 1898, xxiii, 10.

[1] Scott, E. L., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1920, xvii, 120.

[2] Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, xlix, 1.

[3] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, cxxxv, 46.

The sugar returned to normal or nearly so after four or five hours. The carbon dioxide returned after one or two hours and then frequently rose above normal. The oxygen remained below normal for a variable period of a few hours.

Convulsions occurred occasionally in some experiments. The blood sugar at this time was always maximal or nearly so. The carbon dioxide, on the other hand, was usually quite low. However a carbon dioxide as low as six volumes per cent has occurred unaccompanied by convulsions.

In contrast with the preceding results of forced exercise, is the following result with a dog that was allowed to run at its own pace in the treadmill. As a result of travelling 28 kilometers in six hours, the blood sugar gradually fell from 131 mg. to 113 mg. per 100 cc.; the carbon dioxide was lowered from 39.64 volumes per cent to 36.55 volumes per cent and the oxygen was at first raised from 22.64 volumes per cent to 23.65 volumes per cent and then fell to 21.56 volumes per cent.

When, however, we worked the dog in the mill turned by hand at a pace which she did not happen to like, she resisted. Although she was made to travel only 575 meters in 17 minutes, the blood sugar increased from 119 mg. to 131 mg. per 100 cc.; the carbon dioxide was lowered from 40.71 volumes to 24.71 volumes per cent and the oxygen increase was very slight (22.68 volumes to 22.96 volumes per cent). It appeared from this experiment that the reaction of the animal was an important factor in determining whether an increase in blood sugar occurred. We, therefore,

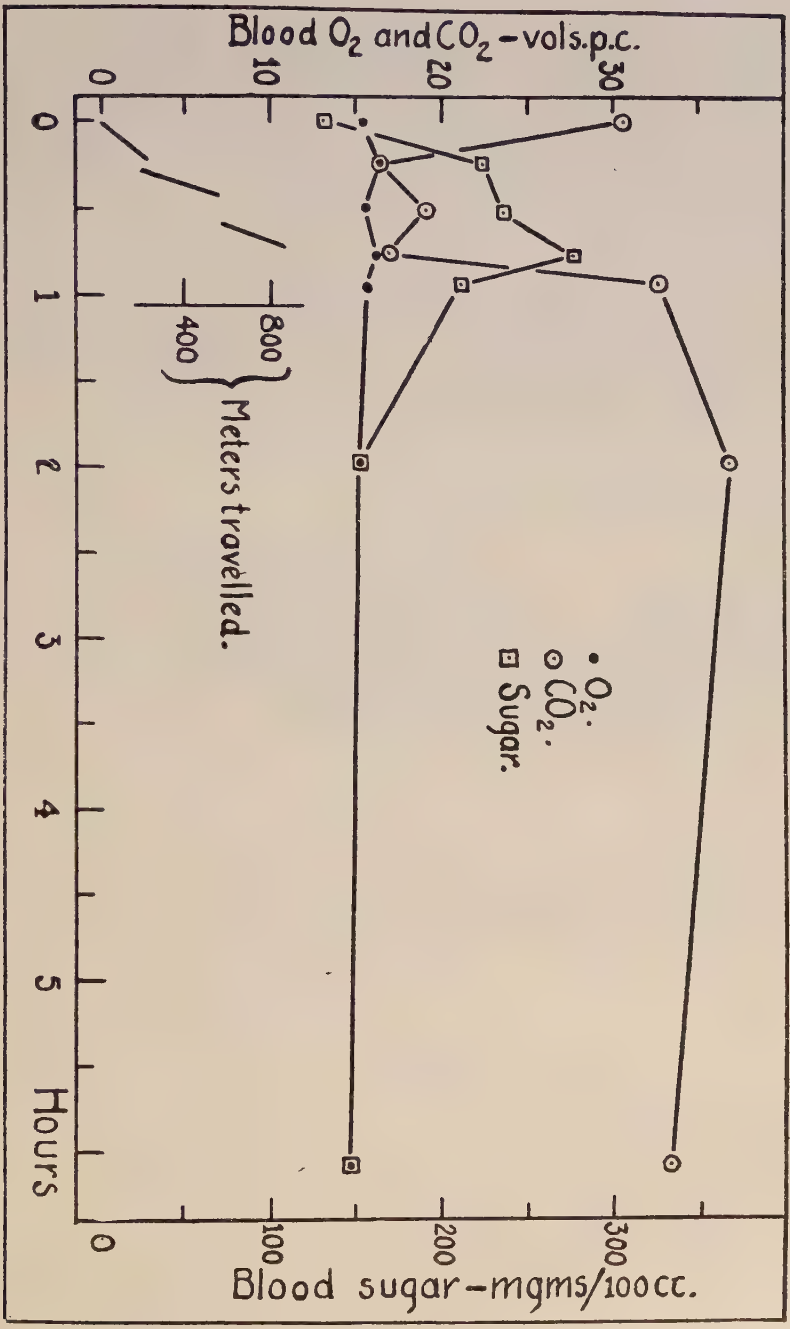
Blood O_2 and CO_2 – vols.p.c.
0
10
20
30
0
1
2
3
4
5
Hours
400
800
Meters travelled.
• O_2.
⊙ CO_2.
⊡ Sugar.
0
100
200
300
Blood sugar – mgms/100cc.

Fig. 1.

trained a cat to run in the treadmill unassisted. This cat had previously shown a great increase in blood sugar and a marked reduction in carbon dioxide when the exercise was forced, *i. e.*, in the hand-driven treadmill (Fig. 1). When running in the treadmill of its own accord, this cat travelled 6090 meters in 160 minutes. During that time the blood sugar increased somewhat, but

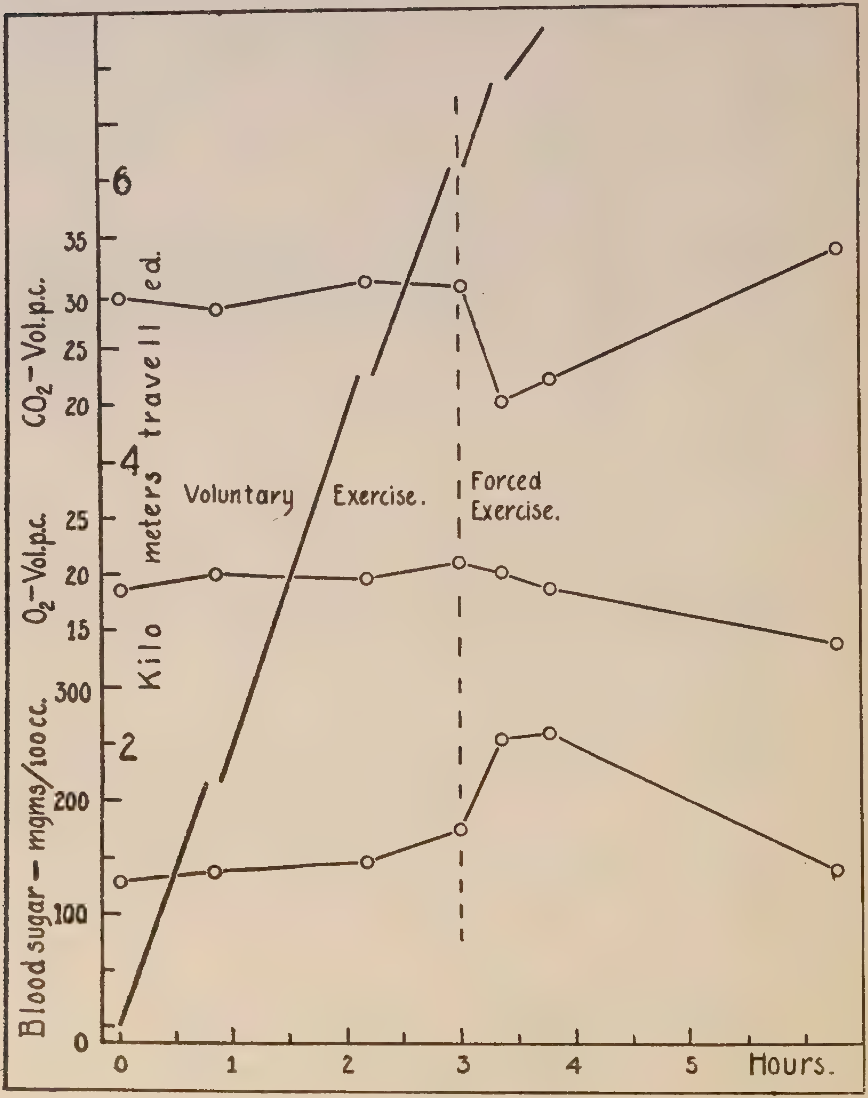

FIG. 2.

nothing like the increase in forced exercise; the carbon dioxide after a slight decrease, increased slightly and the oxygen increased (Fig. 2). The treadmill was then turned by hand. The cat, although travelling no faster than before, resisted by trying to cling and jump part of the time. Although it was made to travel only 960 meters in this manner, the blood sugar increased markedly; the carbon dioxide was much diminished, and the oxygen fell off slightly.

These experiments indicated that the development of aggressiveness and a fighting reaction is important in producing an increase in blood sugar. The carbon dioxide decrease is accounted for by the excessive ventilation (shown by the very great increase in the rate of respiration) accompanying such forced exercise.

282 (2514)

A new method for the colorimetric determination of small concentrations of thymol.

By GEORGE W. PUCHER and LILLIAN A. BURD.

[*From the Laboratories of the Buffalo General Hospital, Buffalo, N. Y.*]

In order to evaluate the thymol content of sodium-fluoride, thymol mixtures used for the preservation of blood samples, the following simple method was devised.

Sulphanilic acid is diazotized and then coupled with thymol in alkaline solution. A reddish brown color stable for at least 50 minutes is obtained. The only alkali that gives satisfactory results is $NaHCO_3$.

METHOD (outline). 3 cc. of a 0.6 per cent sulphanilic acid solution (contains theory HCl necessary to form sulphanilic acid hydrochloride) and 0.5 cc. of 2.0 per cent HCl are pipetted into a sugar tube (Myers-Benedict). 0.5 cc. of a 1.5 per cent aqueous solution of $NaNO_2$ are then added and after one minute 1 to 5 cc. of the thymol solution to be analyzed. (The number of cc. of thymol solution used is diluted so that it will not contain more than 0.5 mg. thymol.) Allow to react 1 minute and then add 2 cc. of a 3.0 per cent $NaHCO_3$ solution. Set aside in diffused light for 10 minutes and then read in a colorimeter against a standard thymol solution 2 cc. of which contains 0.2 mg. (or 0.5 mg.) thymol. Thymol solutions are best prepared by first dissolving the thymol in a small volume of alcohol and then diluting to volume with distilled water.

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* Introduced by H. U. Williams.

In order to obtain the best results by this method the reagents listed above must be prepared and used with an accuracy of at least ±5.0 per cent.

283 (2515)

An undescribed constituent of semen.

By PIERRE A. FISH.

[*From the Department of Veterinary Physiology, Cornell University, Ithaca, N. Y.*]

In the study of spermatozoa with the darkfield microscope in April, 1920, my attention was attracted by the presence of very many minute particles which exhibited the Brownian movement. A number of specimens were examined, always with the same result, except that in perfectly fresh specimens of semen the Brownian movement was not distinctly apparent. In these specimens the particles seemed to be imbedded in a gelatinous matrix, so that the particles moved only when the matrix was moved by the penetrating or lashing movements of the spermatozoa. After some hours the gelatinous condition disappeared and liquefaction supervened. In this latter phase the Brownian movement of the particles became very active. If the cover glass be surrounded with a ring of oil to prevent drying, the particles and their movement may be observed for several days.

Up to date, the particles have been found to be constantly present in the seminal secretion of man, stallion, bull, boar and the testicular fluid of the rooster.

Counts were made of the number of particles by the use of the ocular micrometer method as devised by S. H. Gage. In man the average ranged from 154 to 190; in the stallion, the number averaged in one instance 156 to 165, in another instance 21 to 35. Samples from five different bulls gave quite variable results. In bull No. 1 the particles averaged from 35 to 53; in bull No. 2, 51 to 127; in bull No. 3, the average was 60; in bull No. 4, 19; and in bull No. 5, the range was from 7 to 20. Some of the specimens showing the lower number of particles were obtained from young bulls which had not been in service long. Whether there is any correlation between the number of particles and the age of the animal; or whether there is possibly some relationship indicating the degree of fertility of the fluid are problems still to be solved.

The term spermatomicrons is suggested as an appropriate name for the designation of these particles.

Similar particles (galactomicrons) have been observed in the milk of a number of different species of animals, but in the examination of the other secretions of the body, saliva, urine and bile, I have not yet been able to find them.

Experiments are in progress to determine the nature of these particles. The indications thus far are that they are not composed of fat, but further work is necessary.

In the examination of available literature no reference has as yet been discovered relative to the presence of these particles in the semen.

284 (2816)

Certain pathological tissue changes in thyroidectomized sheep.

By S. A. GOLDBERG and SUTHERLAND SIMPSON.

[*From the Departments of Comparative Pathology and Physiology, Cornell University, Ithaca, N. Y.*]

Of ten lambs that were thyroidectomized early in life, five showed marked lesions. The others were either operated on too late in life or were too young at the time of death. The duration varied from 8 months 4 days to 2 years 5 months after the removal of the thyroids. The lesions were subcutaneous and sub-serous edema with, in most cases, ascites and hydrothorax. The kidneys showed parenchymatous nephritis with hyaline casts in the collecting tubules. The most striking changes were in the thoracic and abdominal aortæ, and in the pulmonary arteries. These showed hyaline degeneration and calcification of the media in the form of placques. These were separated by thickened areas containing numerous fibroblasts and devoid of elastic fibres. Some of the smaller vessels near the aorta showed proliferation of the intima. There was a striking absence of fatty degeneration in any of the vessels. The controls, usually twin lambs of the same sex, did not show any of these tissue changes.

Scholz (1906) describes general edema and the kidney lesions in human cretins, without mentioning the aorta or pulmonary arteries.

Von Eiselsberg (1895) describes the changes in thyroidectomized sheep as being calcification of the intima without fat.

Experimentally, arteriosclerosis is generally produced on a mechanical basis. In this case it seems to be more of a chemical nature, perhaps due to senile changes occurring early in life as a result of complete thyroidectomy.

(The expenses of this research were defrayed by a grant from the Heckscher Foundation of Cornell University.)

285 (2817)

The reason for failure to obtain growth of an obligatory anaerobe (Actinomyces necrophorus) on plate cultures incubated in an anaerobic jar.

By WILLIAM A. HAGAN.

[*From the Department of Comparative Pathology and Bacteriology, New York State Veterinary College, Cornell University, Ithaca, N. Y.*]

In their work on *Bacillus (Actinomyces) necrophorus,* Mohler and Morse[1] reported difficulty in inducing the organism to grow on plate cultures incubated anaerobically although they were successful in cultivating it on the same media in other ways. They do not discuss the cause of the difficulty.

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[1] Mohler, J. R., and Morse, G. B., Bull. No. 167, B. A. I., U. S. Dept. of Agric., 1905.

Recently, in attempting to plate out some cultures of this organism we have experienced the same difficulty. In "hormone" agar shake cultures, good, though not vigorous, growth was easily obtained, but when the same medium was inoculated and poured into Petri dishes and incubated in a Brown anaerobic jar, failure to obtain growth has nearly always resulted.

In preparing the plate cultures, the inoculated medium necessarily is exposed to the air from the time it is poured into the plates until anaerobic conditions become established in the jar. Since the ratio of exposed surface to the volume of the medium is large, there is opportunity for the dissolving of a considerable volume of atmospheric gases, and it was thought that in this fact the cause of the failures might lie.

To test this hypothesis, fluid cultures were exposed to the air in shallow layers in flasks, and subcultures into cooked meat medium were made at intervals. It was found that, although most of the subcultures grew, there always was a period of bacterial lag, the duration of which varied directly with the time of the exposure up to 3 or 4 hours. From this time up to 8 hours, which is the limit of our observations, very little difference was noted in the subcultures. Whereas one loopful of young, normal culture sufficed to give, regularly, a vigorous subculture within 18 hours, a similar amount of the same culture exposed for 30 minutes to 1 hour gave subcultures showing a bacterial lag of 24 to 36 hours or more, and some cultures exposed for 3 to 4 hours have shown lag periods as long as 96 hours.

Cooked meat medium is the most favorable substrate for the cultivation of *Actinomyces necrophorus* which we have found. It is far superior to the plating mediums which we have used in supporting and encouraging growth and in addition, as we shall show in the following paper, it is peculiarly fitted for correcting abnormal oxidation-reduction balances. When subcultures were made from cultures exposed to air into plain infusion bouillon, a medium more nearly like the plating mediums than is cooked meat medium, it was found that growth occurred only occasionally when the exposure was as long as 30 minutes and as much as 0.1 cc. was used as an inoculum. In longer exposures growth failure was almost invariable.

The hypothesis that exposure of the medium to air was the cause of the plating failures now seemed likely to be correct. Direct tests to prove this point were made. The experiments follow:

(a) Plates of "hormone" agar, using about 30 cc. per plate, were poured. With as little delay as possible they were placed in the anaerobe jar and anaerobiosis established. Good growth was obtained after 72 hours. All of the colonies were located in the lower third of the agar layer. Plates containing about 12 cc. of the same agar, inoculated with the same quantity of culture and incubated in the same jar failed to show growth.

(b) Plates of "hormone" agar were poured and the surfaces flooded with petrolatum as quickly as the agar congealed. After incubation in the anaerobe jar, good growth was obtained. Plates without the petrolatum in the same jar failed. Plates with the petrolatum seal, incubated aerobically, failed.

The data indicates that the anaerobic plating failures were due to injury of the bacterial cell forming the inoculum, by reason of their contact with air, so they were not able to multiply in a medium which was not highly favorable but which, nevertheless, supported growth readily from an uninjured inoculum. The following paper shows that the cause of the cell injury is probably due to the formation of hydrogen peroxide by the bacteria while under aerobic conditions.

286 (2818)

The formation of hydrogen peroxide by an obligatory anaerobe (Actinomyces necrophorus). The tolerance of this organism for peroxide.

By WILLIAM A. HAGAN.

[*From the Department of Comparative Pathology and Bacteriology, New York State Veterinary College, Cornell University, Ithaca, N. Y.*]

When difficulty was experienced in obtaining growth of *Actinomyces necrophorus* after the culture had been allowed to come in contact with air (see previous paper), the work of McLeod and Gordon, of Callow, and of Avery and his associates, dealing with hydrogen peroxide production by bacteria immediately came to mind. Using the benzidine test with a bit of raw potato to supply the necessary oxidases, as suggested by Avery in his work on the *Pneumococcus,* the production of peroxide by this organism when in contact with air was readily demonstrated. After exposure to the air in a shallow layer, distinct positive tests for peroxide were obtained in from 1½ to 2 hours in plain bouillon cultures which had been grown under petrolatum seal for 24 to 48 hours. The fluid part of cooked meat cultures, when fairly free from meat fragments, usually gave a positive reaction after two hours exposure but if a considerable quantity of meat fragments was present the reaction was delayed or prevented. When meat fragments were present, the reaction, if it occurred at all, could be obtained over only a short period of time. In most cases a reaction could not be obtained after 8 hours under such conditions, whereas in plain bouillon it could sometimes be obtained for as long as 24 hours.

The inhibiting effect of the meat fragments suggested the presence of catalase. It was also found that when titrated solutions of hydrogen peroxide were introduced into sterile meat medium, the peroxide rapidly was destroyed, or combined in some way so that it would not react to benzidine. The introduction of 1 cc. of a 3 per cent solution of hydrogen peroxide through a melted petrolatum seal and the rapid congealing of the seal formed a means of detecting small amounts of gas formation readily. In this way it was shown that the meat medium actually possessed some oxidizing substance, while the plain bouillon did not. From ¼ to ½ cc. of gas always appeared below the seal in the first case, but there was none in the latter. The cooked meat medium had been sterilized at 120° C. for 1 hour.

A rough idea of the concentration of peroxide formed by the bacteria was obtained by making up various dilutions of titrated hydrogen peroxide in sterile media and comparing the color reactions of the benzidine test with those obtained in the cultures. It was found that, in bouillon, benzidine would detect 1 part of hydrogen peroxide in 100,000 to 200,000. The reactions from the cultures generally were much stronger, at the height of the reaction, than the reaction obtained by the 1-100,000 dilution but not as strong as that obtained in a concentration of 1-10,000.

In meat medium dilutions of hydrogen peroxide as low as

1-1,000 were rapidly destroyed by the meat fragments so that practically no inhibition was noticed in subcultures. Subcultures from tubes in which the meat fragments had been visibly bleached by the peroxide grew readily.

In plain bouillon, however, distinct inhibition was noticeable. Hydrogen peroxide in dilutions of 1-10,000 and 1-100,000 was added to 24 hour cultures. In the 1-100,000 dilution, some lag was noted in subcultures, this period being in proportion to the length of time the organism was subjected to the action of the peroxide. All subcultures, made at intervals up to 6 hours, were growing at 48 hours. In the 1-10,000 dilution the lag period extended from 24 hours to 96 hours, the longer lag periods being in those cultures which were made after longer contact with the peroxide.

In the previous paper a record of the behavior of fluid cultures of this organism after exposure to air in shallow layers was made. This behavior was practically duplicated by those cultures which were in contact with a 1-10,000 dilution of hydrogen peroxide.

287 (2819)

The effect of thyroxin on the cutaneous system in the sheep.

By SUTHERLAND SIMPSON.

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On January 11th, of this year, a thyroidectomized lamb, about eight months old, showed great muscular depression and in order to save its life the subcutaneous administration of thyroxin was begun. Half a milligram was injected every second day for one week; then as recovery began the dose was reduced to ¼ mg. and later to ⅛ mg. In about ten days the animal showed great improvement as was anticipated but after about a month's treatment it was observed that the wool was beginning to fall out in patches and this effect was not expected. The shedding of the wool was first noticed on February 7th; photographs were taken every second or third day to show the progress of the denudation and by February 27th—in about 20 days—the whole animal was bare to the skin with the exception of the head and neck. Considerably later the wool from this area also was shed.

To find out whether thyroxin would have the same effect on the normal sheep, two were selected and each received ¼ mg. subcutaneously every second day beginning on February 13th. In about a month one of these reacted as the thyroidectomized lamb had done, although the shedding of the wool was not so extensive, but the other showed no effect, although later the dose for this animal was increased to 1 mg. every second day and continued till April 27th.

Following thyroidectomy the fleece becomes coarse and ragged giving the animal a tattered, unkempt appearance but the complete and rapid denudation produced by the subcutaneous injection of thyroxin has not been observed in our flock to follow removal of the thyroids. A more detailed statement will follow.

ABSTRACTS OF COMMUNICATIONS.

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288 (2820)

Some new observations concerning the effects of dilute acids and alkalies on proteins.

By HSIEN WU and DAISY YEN.

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Although it has been long known that proteins are subject to changes commonly called denaturation whereby their solubility is altered, our knowledge of the nature and the products of these changes is very limited.

The present study of the effects of dilute acids and alkalies on proteins originated from an observation made by one of us[1] some time ago, that serum proteins which had been dissolved in dilute NaOH solution gave more color with the phenol reagent of Folin and Denis than the same proteins which had not been dissolved in NaOH. With the hope of developing a method based on this observation for the differentiation of proteins, a study of the change in chromogenic value of a number of proteins, both in HCl and in NaOH solutions, was undertaken. Changes of chromogenic value were observed in so dilute solutions of acids and alkalies that no question of the decomposition of the protein could arise, and it occurred to us that the phenomenon under observation may be related to the process of denaturation of proteins. Since there has been no investigation on this subject in recent years, it has seemed desirable to study not only the change in chromogenic value but also other effects which dilute acids and alkalies may have on proteins.

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[1] Wu, H., *J. Biol. Chem.*, 1922, li, 33.

We have studied the problem from three points of view:

1. Change of solubility as evidenced by the formation of a precipitate upon neutralizing the solution.
2. Increase in the amount of color obtained when a definite amount of the protein solution is allowed to react with the phenol reagent of Folin and Denis in the presence of sodium carbonate.
3. Liberation of non-protein substances which can be determined after the removal of the protein.

The routine procedure of our experiments was as follows: An approximately 1 per cent protein solution made faintly acid to methyl red was mixed with an equal volume of N/10 HCl or NaOH, giving a solution about 0.5 per cent in protein and 0.05 N in acid or alkali. The same solution was used for the study of all the three effects mentioned above.

For the study of change of solubility, 100 cc. portions of the solution were measured out and neutralized at different intervals with 50 cc. N/10 NaOH or HCl, as the case may be. The precipitate formed was filtered off and the protein which remained in the filtrate was determined by the Kjeldahl method.

For the study of chromogenic value 1 cc. portions were measured into 50 cc. Erlenmeyer flasks, and at different intervals, diluted with 20 cc. H_2O followed by 0.5 cc. of phenol reagent and 3 cc. of 20 per cent Na_2CO_3. The color thus developed was compared with that of a solution prepared at the same time and in a similar manner from 1 cc. of the original protein solution which had been diluted with an equal volume of H_2O.

For the study of non-protein substances, 100 cc. portions of the solution were measured out, and at different intervals, neutralized with 5 cc. N HCl or NaOH as the case may be; 10 cc. each of 10 per cent sodium tungstate and 2/3 N sulfuric acid were then added and the precipitated protein filtered off. Convenient amounts of the filtrate were taken for the determination of ammonia and chromogenic substances. For the determination of the latter substances tyrosine solutions were used as standard.

The results of our investigation are as follows:

1. All the albumins and globulins which we have studied show change of solubility in 0.05 N HCl or NaOH. The list of these proteins includes the albumins of the egg white of hen, pigeon, goose, duck, serum globulins and albumins of sheep and horse, and the globulin of hempseed, edestin. The solubility of gelatin, proteose and peptones were not changed by dilute acids and alkalies.

2. The velocity of denaturation measured by the change of solubility varies with different proteins. In solutions containing about 0.5 per cent protein in 0.05 N HCl or NaOH the time required for half denaturation of some of the proteins is as follows:

	HCl	NaOH
Hen egg albumin	9 hours	2 hours
Goose egg albumin	3 days	1 minute
Duck egg albumin	2(?) days	2.7 minutes
Pigeon egg albumin	3 hours	——
Horse serum euglobulin	6 minutes	4(?) seconds
Horse serum albumin	——	15 minutes
Edestin	very rapid	very rapid

3. The hydrogen ion concentration decreases in acid protein solution and the hydroxyl ion concentration decreases in alkaline protein solution. This, we believe, is due to an increase in the acid and base binding power of the protein.

4. The velocity of denaturation increases with increasing hydrogen ion concentration or hydroxyl ion concentration. An exception is found in horse albumin which remains unchanged in 0.05 N HCl although it can be denatured in 0.02 N HCl.

5. The velocity constant calculated on the assumption that denaturation is a monomolecular reaction, falls off rapidly with increase in time. Three explanations are offered: (1) the decrease in hydrogen ion concentration or hydroxyl ion concen-

tration already mentioned; (2) only a part of the original protein molecule forms insoluble product, while the remainder is soluble; (3) the insoluble protein undergoes further change into soluble products.

6. All the proteins which show change of solubility also show increase in chromogenic value in 0.05 N NaOH or HCl solution. Gelatin, proteose and peptone show no increase of chromogenic value.

7. The form of the chromogenic value-time curve resembles closely that of the denaturation-time curve.

8. The increase in chromogenic value is not due to the formation of new reactive groups towards the phenol reagent, but rather to an increase in the reactivity of protein molecule so that more groups take part in the reaction. This increase in reactivity can be explained by the protein molecule becoming smaller.

9. The precipitability of the protein by complex acids (*e. g.*, phenol reagent) decreases as the chromogenic value increases.

10. The chromogenic value of the protein denatured by alkali is different from that of the same protein denatured by acid.

11. Ammonia and non-protein chromogenic substances which are probably tyrosine and tytrophane are liberated from the proteins in 0.05 N NaOH at a measurable rate, but only very slowly in 0.05 N HCl solution. The course of liberation of these substances does not run parallel with those of change of solubility and of chromogenic value. The curve for the former change is nearly linear when those for the latter have come practically to a standstill.

12. Hydrogen sulfide is split off from the proteins in alkaline solution but not in acid solution. H_2S forms an inappreciable portion of the non-protein chromogenic substances.

From the above mentioned facts the following conclusions may be drawn:

1. The change in solubility and the increase in chromogenic value of proteins are the results of the same underlying reaction which is commonly called denaturation.

2. The velocity of denaturation, being different for different proteins, should prove of great value for the differentiation of proteins.

3. The underlying reaction of the denaturation of proteins by dilute acids and alkalies is a hydrolysis, probably of some especially labile bonds.

4. The liberation of non-protein substances, NH_3, H_2S and other chromogenic substances is not an essential feature of the denaturation of the protein.
5. The products of denaturation by acids and by alkalies are different.

289 (2821)

The application of the eosin-criterion for the viability of protozoan cysts to cysts of Hartmanella hyalina treated with chlorine-water.

By JOHN F. KESSEL.

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Owing to the fact that no satisfactory method has been developed to induce the excystment and growth of the amebæ parasitic in the human intestinal tract, no absolute criterion has been established to distinguish the viable from the non-viable cysts. The penetration of the cysts by analine dyes is regarded as an indication that the cysts are dead and the use of eosin as an indicator has been generally accepted. The ultimate fate of the cysts which fail to take the eosin stain has not been finally determined. It has been shown that cysts which undergo a plasmolysis may not stain red by the eosin, though they are considered to be incapable of development.

The present investigation was undertaken in order to determine the percentage of free chlorine in water necessary to retard the development of cysts of *Hartmanella hyalina* and at the same time to determine the fate of the cysts which presented an apparently normal appearance. It is hoped that by comparing the resistance of the cysts of the human intestinal Protozoa with the resistance of the coprozoic ameba under consideration that information may be procured regarding the relative value of methods employed in disinfecting fruits and vegetables for table use and regarding the general resistance of Protozoan cysts.

Cysts of *Hartmanella hyalina* were placed in water containing different percentages of free chlorine in solution. A ten minute interval was allowed before the cysts were transferred to a suitable culture medium which in this investigation was 10 per cent horse serum in Locke's solution diluted with 50 per cent distilled water. The cysts were counted at the time of their transfer to the culture medium and the reaction of a representative number of the cysts to Donaldson's iodine-eosin was noted. The cultures were kept under observation until all possibility for further excystment had occurred. Tests were made with sixty-four different cultures which had been placed in chlorine-water, the percentages of free chlorine which ranged from .006 to 4.

The following general conclusions may be drawn:

1. Two per cent of free chlorine in water is the strongest solution which will permit excystment and development of cysts of *Hartmanella hyalina* after having been exposed to the solution ten minutes. This is approximately the amount of free chlorine liberated by placing 32 gm. or eight teaspoonsfull of chlorinated lime in one pint of water.
2. Red cysts and plasmolyzed cysts do not undergo excystment and development.
3. Partially plasmolyzed cysts may, after a delayed period, take the eosin stain but the greater the degree of plasmolysis, the less likelihood there is for the penetration of the eosin.
4. All cysts which appear normal at the time of transfer and assume the green color produced by the iodine are not viable, but some may, after a period, ranging from a few hours to 72 hours, stain red by the eosin.
5. This lag period varies according to the concentration of the chlorine water used and probably also varies according to the resistance of the cysts in question and according to the species involved. It should be considered in future experimental work on the resistance of Protozoan cysts where the eosin-criterion is used for determining viability.

290 (2822)

A new instrument and operation for aseptic end-to-end intestinal anastomosis.

By JEROME P. WEBSTER (by invitation).

[*From the Department of Surgery, Peking Union Medical College, Peking, China.*]

An instrument, employed successfully in 86 operations on dogs and one on man, was devised in 1921 for cutting purse-string ligatures about the occluded stumps of intestine after the ends had been abutted and enterorrhaphy practically completed. It consists of (1) a cylindrical steel needle with a round eye at one end for the ligature, (2) a tubular knife of two pieces joined by threads, and (3) a tubular thumb-cap with thumb-screw at one side. The knife is revolved and passes down over the eye of the needle when the latter is inserted in the tubular knife and held at its other end by the thumb-cap with the thumb-screw acting as a vise.

After cutting the mesentery and ligating the vessels, the bowel is doubly clamped slightly obliquely with Kocher clamps on each side of the loop to be resected, and the loop is cut away between clamps with electric cautery or by cutting and carbolizing. On either side of the clamps at about 1 cm. distance, purse-string sutures of catgut or silk are laid about the bowel with the ends left free opposite the mesenteric border. The clamps are approximated and six mattress sutures of fine silk or catgut, which pick up the submucosa, are laid but not tied between the bowel loops, coming 2 to 3 mm. from the purse-string sutures. The bowel loops are placed parallel. The cylindrical needles are threaded on one of the loose ends of each purse-string suture, and the latter are tied and cut. The bowel is severed just beyond the purse-string sutures and the stumps are cauterized. The cut ends are abutted, the mattress sutures are tied and reenforced with intermediate sutures. All are tied except those about the needles at the free border. The tubular knives are sheathed over the needles. The thumb-caps are applied over the ends of the needles holding them tightly by the thumb-screws. The tubular knives are revolved cutting the purse-string sutures, and the knives are withdrawn. The last mattress suture is tied and the mattress sutures are pulled outwards radially opening up the lumen. The rent in the mesentery is then sutured.

During 1922, a series of anastomoses on the small bowel using this instrument, was performed on dogs, tying off the catgut sutures and severing the bowel before suturing the apposed ends together with mattress or other types of sutures. These sutures were placed as closely as possible to the bowel ligatures to reduce the diaphragm. The lumen was not opened at operation but peristalsis was depended upon to re-establish the lumen. Difficulty was encountered in placing the sutures evenly and 40 per cent of the dogs died as a result of the operation. Other dogs sacrificed showed clinical symptoms of partial obstruction and had more or less dilatation of the proximal loops.

Twenty-eight consecutive anastomoses were performed by the technique first described. Specimens were recovered from zero hours to 44 days after operation. One ileo-colostomy and two colo-colostomies were performed in this series, the remainder being on the small bowel. Two dogs died from penumonia and one the 21st day from intussusception below the anastomosis, but with a satisfactory lumen and no adhesions. The diaphragm was satisfactorily opened. Solid food, including bones, was given 24 hours after operation. There was but little adhesion formation about the suture line in these cases and clinically no symptoms of obstruction.

Another series was performed according to Bidgood's modification of Halsted's operation. Three operations were on the large bowel. One dog died on the 63rd day from other causes. One died on the 15th day from pneumonia but showed complete blocking of the lumen by a large diaphragm. The third dog was sacrificed after operation. Of three operations on the small bowel, one died of marked intestinal obstruction the third day, and another died on the second day following operation to relieve marked intestinal obstruction. In the third operation Bidgood's technique was followed, but the mattress sutures were taken as closely as possible to the purse-string sutures. At operation 2 days later, moderate dilatation of the proximal loop was found.

Another series of anastomoses, according to the author's technique, was performed. Of nine consecutive operations, one dog died five hours after operation to relieve intestinal obstruction caused by Bidgood's operation. One died the 41st day from marked distemper. The remaining dogs were operated upon or sacfificed, recovering specimens from 33 to 107 days after operation. Three of these anastomoses were incidental to other operations. Double zero catgut mattress sutures on welded needles were used throughout. There were no symptoms of obstruction, no dilatation nor constriction from the diaphragm and no adhesions about the bowel as a result of anastomosis.

CONCLUSIONS.

An instrument has been devised for cutting purse-string sutures used with satisfaction and precision in 87 operations. It cuts without tension on the suture material; it is simple; it can be sharpened readily and repeatedly; the needles are light and do not pull on the purse-string sutures.

Twenty-eight consecutive operations with removal of specimens from zero hours to 44 days, have been performed with a minimum of adhesions with no signs of obstructions and with no death that could be attributed to the operation. Eight consecutive operations with specimens recovered from 33 to 107 days have been performed without a death from the operation, and with no adhesions. In this type of anastomosis on the small bowel, the sutures must be taken close to the purse-string sutures and the lumen should be opened at operation. Aseptic end-to-end anastomosis of the bowel can be safely and easily performed by this method.

291 (2823)

Healing of aseptic end-to-end intestinal anastomoses by the author's method.

By JEROME P. WEBSTER (by invitation).

[*From the Department of Surgery, Peking Union Medical College, Peking, China.*]

A study was made of specimens of 86 end-to-end anastomoses performed on dogs using a revolving tubular knife for cutting ligatures or purse-string sutures placed about the closed abutted ends of severed bowel after enterorrhaphy was practically complete. Several types of silk or catgut sutures were used. Stitches approximating the serous surfaces were laid at various distances from the occluding ligatures or purse-string sutures beyond which, at different lengths, the bowel was severed by electric cautery or by cutting and carbolizing. In some cases the serous and muscular coats were stripped back and the ligatures were placed about the submucosa or near the cut edges. The bowel was cut perpendicularly to the long axis, slightly obliquely, or at an angle of 45°.

Rapidity of healing depends upon (1) the amount of trauma or necrosis produced by severing the bowel and sterilizing the cut ends; (2) the proximity of the cut mucosal surfaces to each other at the tip of the inturn; (3) the position and length of the apposed bowel walls at the inturn; (4) the vascularity of the inturn which largely depends upon the size, type, material and tightness of the sutures, and the angle at which the bowel is severed, and (5) the amount of infection about the area of anastomosis.

Cutting and carbolizing the stumps causes less extensive trauma, necrosis and hemorrhage to the bowel wall than does cauterizing thermally although, unless proper precautions are taken, there is more chance for soiling by the former method. Stripping back a sero-muscular cuff and ligating the mucosa and submucosa hastens healing but adds an unnecessary step to the operation.

The inturned walls agglutinated by fibrinoplastic exudate by the completion of the operation and they begin to retract immediately on being freed from their normal tension. If the inturn is short the line may be practically straightened by the 8th day and completely straightened by the 21st day, but such rapid straightening is inadvisable. Other specimens show very slight thickening at the line of anastomosis by the 105th day. The size of the diaphragm largely determines the length of time for the inturn to straighten out. When the diaphragm is very small the bridge is composed of a broad band of connective tissue. Two layers of sutures delay the straightening of the inturn.

When the mucosal surfaces are in close proximity with slight destruction and good blood-supply the epithelium can be seen starting to cross the gap 48 hours after operation and it may be entirely covered across by the 7th day. The line of epithelium is of the embryonic, low cuboidal, undifferentiated type which may grow down from the surface of embryonic villi or out from the glands. By the 21st day there is a thick covering of mucosa everywhere and by the 33rd day it has become normal in size and shape.

The *stratum fibrosum* and *granulosum* stretch across the gap made by the cut and the *stratum fibrosum* receives fibres from the submucosa and new connective tissue at the apposed surfaces. The *muscularis mucosæ* is found practically normal by the 68th day. The fibres join from either side and may receive fibres from the inturned longitudinal muscle.

The circular muscle is separated by a more or less thin line of connective tissue across which the muscle fibres never pass. The longitudinal muscle gradually disappears from the inturn, being replaced by connective tissue, but grows across from either side under the serosa, forming a continuous band at least by the 68th day.

The fibrinoplastic exudate becomes invaded with fibroblasts by the 3rd day and strong connective tissue bands are formed. These gradually thin out to narrow fibrous bands by the 107th day.

Silk sutures not entering the lumen may be drawn to one side during the process of the straightening of the inturn and may be found by the 104th day lying quietly without reaction beneath the serosa. Sutures entering the mucosa are walled about with leucocytes and a new epithelial layer from the glands begins to surround them by the 2nd day. *Stratum granulosum, stratum fibrosum* and *muscularis mucosæ* grow about the epithelial layer to restore the gland to its normal position. Silk sutures are found on the 68th day without reaction and extending from beneath the serosa out into the lumen. They may be found still hanging in the lumen by the 104th day. The No. 1 catgut purse-string sutures are absorbed or cast off into the lumen and are not found after the 7th day.

There is dilatation of the blood vessels in the inturn beginning within the first 22 hours and remaining until the 27th day. Tight sutures may cause anemia or infarction in the inturn thereby delaying healing. Sutures at mesenteric border do not threaten the blood supply at the free border if the bowel is cut slightly obliquely. Catgut sutures on ordinary needles cause more trauma and hemorrhage than those on welded needles. One layer of Halsted mattress sutures, when properly placed, is sufficient for safe end-to-end intestinal anastomosis.

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2379. [with **V. M. Hosepian, J. R. Enright** and **Dorothy F. Porter.**] Reactions of the urinary bladder in canine anaphylaxis.

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2443. [with **W. S. Hastings** and **C. Anastasia.**] A paradoxical stability phenomenon with spontaneously agglutinating diphtheria bacilli and its practical import.

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2276. [with **Lafayette B. Mendel** and **Edwards A. Park.**] Experimental production of rickets with diets of purified food substances.

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2343. [with **Harry H. Knight.**] Carotin in *Perillus bioculatus* (Fab.) and its derivation from the lymph of *Leptinotarsa decemlineata* (Say).

2344. [with **H. H. Knight.**] Anthocyanin and flavone-like pigments in phytophagous and predaceous forms of hemiptera.

2502. [with **E. Samuelsson.**] The nature of the substances adsorbed on the surface of the fat globules in cow's milk.

Papanicolaou, G. N.

2315. [with **N. F. Blau.**] The ovarian cyst fluid with special reference to its effect upon the reactions of the genital tract.

2431. Ovogenesis during sexual maturity as elucidated by experimental methods.

Pappenheimer, Alwin M.

2303. [with **Louise D. Larimore.**] The occurrence of gastric lesions in rats and their possible relation to dietary deficiency.

2484. The prevention of experimental low-phosphorus rickets in rats by the subcutaneous administration of potassium phosphate.

Park, Williams H.

2426. See **Zingher, A.**

Park, Edwards A.

2276. See **Osborne, Thomas B.**

Parker, Julia T.

2489. [with **Caroline S. Finley.**] Some results obtained by the treatment of nervous phenomena with glucose.

Pearce, Louise.

2396. [with **C. M. Van Allen.**] Operative results of thyroidectomy and thymectomy in the rabbit.

2420. See **Brown, Wade H.**

2421. See **Brown, Wade H.**

Perlzweig, William A.

2244. [with **Lois Wells** and **Harold L. Amoss.**] A note on the antigenic properties of the filtrate of typhoid saline vaccine.

2247. [with **Emily Latham** and **Chester S. Keefer.**] The behavior of inorganic phosphate in the blood and urine of normal and diabetic subjects during carbohydrate metabolism.

Piper, H. A.

2403. See **Murlin, John R.**

Plummer, M. B.

2424. [with **H. J. Deuel, Jr.,** and **G. R. Cowgill.**] The rôle of the liver in pancreatic secretion.

Pohlman, A. G.

2402. [with **F. W. Kranz.**] Binaural minimum audition in a subject with ranges of deficient acuity.

Porter, Dorothy F.
2379. See **Manwaring, W. H.**
2501. See **Manwaring, W. H.**
Proctor, Elmer E.
2432. See **Fried, B. M.**
Pucher, George W.
2289. See **Cori, Carl F.**
2514. [with **Lillian A. Burd.**] A new method for the colorimetric determination of small concentrations of thymol.

Rakestraw, Norris W.
2234. [with **Florence O. Whittier.**] The effect of loss of sleep on the blood and urine.
2300. [with **Robert E. Swain.**] The uric acid content of the blood of the Stellar sea-lion.
Rask, O. S.
2498. See **Alsberg, C. L.**
Richardson, H. B.
2461. See **Tolstoi, E.**
Riley, P. J.
2297. See **McClendon, J. F.**
Robertson, O. H.
2250. [with **Richard H. P. Sia** and **Shutai T. Woo.**] The protective action of gelatin for pneumococci suspension.
2287. [with **Shutai T. Woo** and **Richard H. P. Sia.**] A method for maintaining stability in reaction of solutions during sterilization.
Robscheit-Robbins, F. S.
2511. See **Whipple, G. H.**
Rockstraw, Elizabeth W.
2462. [with **J. G. Hopkins.**] Zone phenomena in the Kahn precipitation test for syphilis.
Rose, A. R.
2236. See **Sherwin, C. P.**
2351. See **Sherwin, C. P.**
Rose, E. J.
2340. See **Beckwith, T. D.**
Rose, Mary Swartz
2304. [with **Grace MacLeod** and **Bertha Bisbey.**] Maintenance values for the proteins of milk, bread and milk, meat and soy bean curd in human nutrition.

Rosenthal, Sanford, M.

2270. A new method of using phenolsulfonephthalein for testing renal function.

2271. A new method of testing liver function with phenoltetrachlorphthalein.

Ross, Victor.

2324. The influence of the ingestion of potassium chlorate, sodium chloride and water on the oxygen capacity of the blood.

Rothwell, Carmen.

2242. See **Kugelmass, I. Newton.**

Rucker, C. W.

2506. See **Hayden, Edward M.**

Rupp, Alice.

2373. See **Visscher, Maurice B.**

Sabin, F. R.

2398. See **Cunningham, R. S.**

2399. [with **C. A. Doan** and **R. S. Cunningham.**] The separation of the phagocytic cells of the peritoneal exudate into two distinct types.

Samuelsson, E.

2502. See **Palmer, L. S.**

Scammon, Richard E.

2342. [with **Halbert Dunn.**] On the growth of the human cerebellum in early life.

2508. [with **LeRoy A. Calkins.**] The relation between body-length and body-weight in the human embryo and fetus.

Schick, B.

2458. [with **Margaret E. Fries, Jerome Kohn** and **Philip Cohen.**] Observations of the nutritional effect of subcutaneous oil injections.

Schield, E. L.

2404. The antiscorbutic value of canned vegetables and its preservation by exhausting the air before processing.

Schmidt, Carl F.

2366. The action of morphine, heroin and codeine on respiration.

2367. The action of respiratory stimulants in morphine and heroin poisoning.

2412. See **Chen, K. K.**

2439. [with **K. K. Chen.**] The influence of experimental ascites on the diuretic action of drugs.

Schmidt, Carl L. A.
2331. See **Greenberg, D. M.**
2336. See **Brakefield, J. L.**
2378. See **Greenburg, D. M.**
Schneider, Edward C.
2448. The gaseous exchange in man during short exposures to a low barometric pressure.
Scott, F. H.
2298. The effect of hemorrhage on the blood after removal of the liver.
2505. See **King, G. L.**
Seago, Dorothy W.
2363. See **Macht, David I.**
Seckinger, Daniel L.
2490. [with **Franklin F. Snyder.**] Cyclic variations in the spontaneous contractions of the human fallopian tube.
Shaklee, A. O.
2477. [with **F. O. Anderson.**] Vomiting reflex from stomach after vagotomy.
Shaughnessy, H. J.
2454. See **Winslow, C.-E. A.**
Sheplar, Adele E.
2233. See **Baylis, Adelaide B.**
Sherman, H. C.
2237. [with **M. R. Grose.**] A quantitative study of the destruction of vitamin B by heat.
Sherman, J. M.
2392. See **Holm, George E.**
Sherman, Lillian.
2325. See **Dochez, A. R.**
Sherwin, C. P.
2236. [with **A. R. Rose.**] Cystine metabolism.
2305. See **Muldoon, J. A.**
2351. [with **A. R. Rose** and **A. Weber.**] Sulfur metabolism. III.
2388. See **Byrne, Joseph.**
Shiple, G. J.
2305. See **Muldoon, J. A.**
Shohl, A. T.
2235. See **Kugelmass, I. Newton.**

Shulman, David N.

2452. [with **Lafayette B. Mendel.**] The blood platelets in rats on adequate and inadequate diets.

Sia, Richard H. P.

2250. See **Robertson, O. H.**

2287. See **Robertson, O. H.**

Simpson, Sutherland.

2377. See **Bodansky, Aaron.**

2516. See **Goldberg, S. A.**

2519. The effect of thyroxin on the cutaneous system in the sheep.

Skinner, Charles E.

2327. [with **T. J. Murray.**] A medium for the inhibition of spreaders and the differentiation of *B. coli* and *B. aerogenes.*

Smith, Arthur H.

2418. See **Koch, Mathilde L.**

Smith, Curtis E.

2333. See **Jones, Martha R.**

Smith, David T.

2430. See **Olitsky, Peter K.**

Smith, Philip E.

2335. [with **A. T. Walker** and **James B. Greaser.**] The production of the adiposogenital syndrome in the rat, with preliminary notes upon the effects of a replacement therapy.

Smyly, H. Jocelyn.

2413. [with **Charles W. Young.**] The experimental transmission of leishmaniasis to animals.

2414. See **Young, Charles W.**

Snyder, Franklin F.

2490. See **Seckinger, Daniel L.**

Speidel, C. C.

2433. See **Jordan, H. E.**

Stevens, Franklin A.

2249. [with **A. R. Dochez.**] The study of hemolytic streptococci associated with scarlet fever.

2273. The rate of growth of virulent and avirulent streptococci in serum media.

Stewart, Harold J.

2422. The use of calcium chloride in edema due to heart failure.

2423. A method for obtaining samples of mixed venous blood in intact dogs.

Strauss, M. L.
2476. See **Benedict, E. M.**
Sumner, James B.
2509. [with **Vi A. Graham** and **Charles V. Noback.**] The purification of Jack bean urease.
Sutter, C. Clyde.
2403. See **Murlin, John R.**
Swain, Robert E.
2300. See **Rakestraw, Norris W.**
Swigart, Margaret.
2381. See **Macht, David I.**
Swingle, W. W.
2313. See **Nicholas, J. S.**
2450. [with **J. S. Nicholas.**] Autoplastic and homoplastic parathyroid transplantation.

Ten Broeck, Carl.
2369. [with **Johannes H. Bauer.**] Tetanus carried in experimental animals.
Thatcher, Harvey S.
2419. The oral administration of insulin to rabbits.
Thomas, A. W.
2319. [with **E. R. Norris.**] Irregular series in protein precipitation.
Thomas, Bernard G. H.
2278. Studies on the pathological effect of organisms of the enteritidis paratyphoid B group on the pancreas, liver and kidneys.
Thompson, H. C.
2446. See **Wilson, D. Wright.**
Thompson, William P.
2415. [with **Frank L. Meleney.**] A comparative method of testing the enzymes of living hemolytic streptococci. I.
Thurlow, Sylva.
2446. See **Wilson, D. Wright.**
Ting-An, Li.
2411. See **Mills, Ralph G.**
Tolstoi, E.
2461. [with **R. O. Loebel, S. Z. Levine** and **H. B. Richardson.**] The production of lactic acid in diabetes following the administration of insulin.

Travell, Janet G.
2473. [with **Jeanette Allen Behre.**] The effect of insulin upon the rate of fermentation of glucose by yeast.
Tso, Ernest.
2437. The value of egg yolk in supplementing diets deficient in calcium.

Van Allen, C. M.
2396. See **Pearce, Louise.**
2420. See **Brown, Wade H.**
2421. See **Brown, Wade H.**
2468. Phenomena of blood coagulation with special reference to a transplanted tumor of the rabbit.
Van Der Scheer, J.
2360. See **Landsteiner, K.**
Visscher, Maurice B.
2373. [with **Alice Rupp.**] The respiratory wave in arterial blood pressure.

Walker, A. T.
2335. See **Smith, Philip E.**
Walzer, Matthew.
2253. [with **Ella F. Grove.**] A study of the anaphylactogenic properties of aqueous extracts of pollens and of Le Page's glue.
Wayman, Marguerite.
2355. See **Mueller, J. Howard.**
Weber, A.
2351. See **Sherwin, C. P.**
Webster, Jerome P.
2522. A new instrument and operation for aseptic end-to-end intestinal anastomosis.
2523. Healing of aseptic end-to-end intestinal anastomoses by the author's method.
Webster, L. T.
2352. I. Experimental rabbit snuffles.
2353. II. Experimental rabbit pneumonia.
Weinstock, Mildred.
2457. See **Hess, Alfred F.**
Weiss, Robert.
2463. See **Deuel, Jr., Harry J.**

Weiss, Soma.
2307. See **Morris, Ruth M.**
2401. See **De Graff, Arthur C.**
Wells, Lois.
2244. See **Perlzweig, William A.**
Wenner, W. T.
2506. See **Hayden, Edward M.**
West, Randolph.
2345. See **Benedict, Ethel M.**
2476. See **Benedict, E. M.**
Wheatley, Marjorie A.
2347. See **Geyelin, H. Rawle.**
Whipple, G. H.
2511. [with **F. S. Robscheit-Robbins.**] Regeneration of hemoglobin and diet factors in prolonged, severe experimental anemia.
Whitsett, J. Wilbur.
2483. See **Allen, Edgar.**
Whittier, Florence O.
2234. See **Rakestraw, Norris W.**
Wible, Charles.
2258. The locus of the action of veratrin in *Mnemiopsis*.
Williams, A. W.
2384. [with **H. D. Hussey** and **E. J. Banzhaf.**] Culture filtrates of hemolytic streptococci from scarlet fever: Intradermal reactions in test animals.
Williams, R. R.
2390. See **Eddy, Walter H.**
Wilson, D. Wright.
2446. [with **W. L. Long, H. C. Thompson** and **Sylva Thurlow.**] Changes in the composition of the urine after muscular exercise.
2447. See **Liljestrand, S. H.**
Winslow, C.-E. A.
2454. [with **H. J. Shaughnessy.**] The alkaline isopotential point of the bacterial cell.
Witt, Dan H.
2429. See **Landsteiner, Karl.**
Witter, Margaret S.
2307. See **Morris, Ruth M.**

Woo, Shutai T.
2250. See **Robertson, O. H.**
2287. See **Robertson, O. H.**

Wright, F. R.
2291. See **Hubbard, R. S.**

Wu, Hsien.
2284. A modification of Duboscq-Pellin colorimeter for bicolorimetric work as in colorimetric pH determination without buffer mixtures.
2520. [with **Daisy Yen.**] Some new observations concerning the effects of dilute acids and alkalies on proteins.

Yen, Daisy.
2520. See **Wu, Hsien.**

Young, Charles W.
2413. See **Smyly, H. Jocelyn.**
2414. [with **H. Jocelyn Smyly** and **Cabot Brown.**] Experimental kala-azar in a hamster (*Cricetulus griseus,* M.-Edw.)

Zau, Zung-Dau.
2365. See **Meleney, Frank L.**

Zeman, F. D.
2322. [with **William Friedman** and **L. T. Mann.**] Kidney changes in pyloric obstruction.

Zingher, A.
2385. Results obtained with the Dick test in normal individuals and in acute and convalescent cases of scarlet fever.
2426. [with **William H. Park.**] Immunity results obtained in school children with diphtheria toxoid (modified toxin) and with 1/10 L + mixtures of toxin-antitoxin.
2427. The significance of the pseudoreaction in the Dick test and methods used for its identification.
2428. The irritating local effect of human blood serum or plasma containing chyle obtained soon after meals.
2486. Further studies with the Dick test and active immunization with scarlet fever streptococcus toxin.

Zinsser, Hans.
2355. See **Mueller, J. Howard.**

Zucker, T. F.
2326. [with **M. J. Matzner.**] On the pharmacological action of the antirachitic active principle of cod liver oil.

EXECUTIVE PROCEEDINGS.

MAIN SOCIETY.

One Hundred Thirty-third Meeting.

New York Post-Graduate Medical School, October 17, 1923. President Jackson in the chair.

Members present: Berg, Dubin, DuBois, Eddy, Harris, I. F., Hastings, Jackson, H. C., Killian, Kingsbury, MacNeal, Myers, Ottenberg, Pappenheimer, Rakestraw, Rose, A. R., Sherman, H. C., Sherwin, Shohl, West, Zingher.

Members elected: W. A. Hagan, H. J. Deuel, Jr., M. Heidelberger.

Resignation: C. J. West.

One Hundred Thirty-fourth Meeting.

Rockefeller Institute for Medical Research, November 21, 1923. President Jackson in the chair.

Members present: Avery, Bailey, C. V., Bauman, L., Berg, Brooks, M. M., Bronfenbrenner, Coca, Cohen, M., Crozier, Du Bois, Deuel, Eggston, Gates, Harris, I. F., Hastings, Hess, Jackson, H. C., Goldfarb, MacNeal, Marine, Moore, A. R., Morse, W., Myers, V. C., Oppenheimer, Olitsky, Ottenberg, Rakestraw, Rose, A. R., Sherwin, Ulenhuth, Van Slyke, Wakeman, West.

Members elected: J. H. Bauer, H. C. Bazett, H. L. Dunn, Carl Hartman, Soma Weiss.

One Hundred Thirty-fifth Meeting.

College of Physicians and Surgeons, December 19, 1923. President Jackson in the chair.

Members present: Bagg, Benedict, Bostrom, Deuel, Dubin, Edwards, Eisberg, Friedman, Hess, Jackson, Jobling, Killian, Myers, V. C., Pappenheimer, Prewitt, Rakestraw, Rose, A. R., Rose, M. S., Sittenfield, Wallace, Weiss, Wollstein, Zucker.

Members elected: C. M. Child, C. F. Cori, E. A. Doisy, J. F. Kessel, K. Landsteiner, P. D. McMaster, I. H. Page, G. N. Papanicolaou, H. B. Richardson, G. J. Shiple, K. Thomas, E. Tolstoi.

Resignation: H. K. Hayes.

One Hundred Thirty-sixth Meeting.

Cornell University Medical College, January 16, 1924. President Jackson in the chair.

Members present: Avery, Baehr, Bailey, C. V., Blake, Danzer, Dochez, DuBois, Edwards, Friedman, Greenwald, Jackson, Jobling, Lusk, Mackenzie, Myers, V. C., Page, Palmer, Parker, Rose, A. R., Tolstoi, Zucker.

Members elected: H. S. Burr, C. A. Doan, Benjamin Harrow, S. C. Harvey, R. C. Lewis, A. H. Morse, Stuart Mudd, C. N. Myers, C. C. Speidel, G. H. Smith, W. C. Stadie, M. C. Winternitz.

One Hundred Thirty-seventh Meeting.

Presbyterian Hospital, February 20, 1924. President Jackson in the chair.

Members present: Bagg, Bailey, C. V., Baumann, E. J., Bauman, L., Clowes, Coca, Dochez, Gates, Geyelin, Goldfarb, Greenwald, Harrow, Jackson, Jobling, Killian, Mackenzie, Myers, V. C., Papanicolaou, Parker, Rose, A. R., Wallace, West, Zucker.

Members elected: J. P. Baumberger, F. A. Csonka, W. S. Ladd, S. A. Levinson, Béla Schick.

One Hundred Thirty-eighth Meeting.

University and Bellevue Hospital Medical College, March 19, 1924. President Jackson in the chair.

Members present: Avery, Banzhaf, Blake, Bostrom, Coombs, Danzer, Dochez, Eddy, Edwards, Eisberg, Epstein, Greenwald, Hess, Jackson, H. C., Killian, Kirkbride, Macht, Myers, V. C., Ottenberg, Park, W. H., Prewitt, Richardson, Rose, A. R., Senior, Stevens, Tolstoi, Torrey, Williams, A. W.

Members elected: J. B. Collip, H. W. Davies, R. G. Green, L. Emmett Holt, Jr., J. H. Korns, C. P. Miller, Bret Ratner, L. T. Webster, Harry Weiss.

One Hundred Thirty-ninth Meeting.
(Twenty-first Annual Meeting.)

College of the City of New York, April 16, 1924. President Jackson in the chair.

Members present: Berg, Brooks, S. C., Brown, W. H., Browne, W. W., Cohn, A. E., Crozier, Csonka, Deuel, Dubin, Eisberg, Goldfarb, Hess, Jackson, Killian, Kleiner, Moore, A. R., Murray, T. J., Myers, C. N., Myers, V. C., Northrup, Pappenheimer, Prewitt, Ratner, Rose, A. R., Rous, Scott, G. G., Sherwin, Sittenfield, Van Slyke, Wohlstein, Youland, Zingher.

Members elected: Edgar Allen, Adolph Bernhard, I. N. Kugelmass, W. G. Penfield, E. W. Schultz.

Officers of the Society elected: Holmes C. Jackson, President; J. W. Jobling, Vice-President; V. C. Myers, Secretary-Treasurer; Peyton Rous, Member of Council.

Nominating Committee for 1925: W. R. Bloor, A. J. Goldfarb, L. B. Mendel, C. P. Sherwin, D. D. Van Slyke, G. B. Wallace, Hans Zinsser.

Endowment Fund: The Treasurer reported that through contributions made by members of the Society, the Meltzer Endowment Fund now amounts to $6806.41.

The annual dinner was held at the College of the City of New York following the one hundred thirty-ninth meeting. The following members were present: Berg, Bauman, E. J., Brooks, S. C., Brown, W. H., Browne, W. W., Crozier, Deuel, Dubin, Eisberg, Friedman, Gay, Goldfarb, Hess, Hooper, Jackson, H. C., Killian, Kleiner, MacNeal, Moore, A. R., Murray, Myers, C. N., Myers, V. C., Northrup, Prewitt, Ratner, Rose, A. R., Rous, Scott, G. G., Sherwin, Van Slyke, D. D., Youland.

One Hundred Fortieth Meeting.

Sterling Hall of Medicine, Yale University, May 24, 1924. President Jackson in the chair.

Members present: Baitsell, Baumann, E. J., Benedict, S. R., Blake, Cowgill, Csonka, Deuel, Dubin, Edwards, Frankel, F. H., Greenwald, Guy, Halsey, Harris, I. F., Hess, Hopkins, Jackson, Jobling, Killian, Kugelmass, Ladd, Lusk, Lynch, Mackenzie, Mendel, Morse, Myers, C. N., Myers, V. C., Packard, Park, E. A., Peters, Ratner, Richardson, Rose, A. R., Schick, Schneider, Shohl, Stadie, Smith, A. H., Swingle, Tolstoi, Van Slyke, Wilson, D. W., Winternitz, Underhill, Zucker.

Members elected: Joseph Byrne, E. P. Carter, Hilda M. Croll, Amy L. Daniels, F. R. Griffith, O. H. Plant, G. G. Ornstein, R. R. Williams.

The meeting was followed by a dinner in Dwight Hall.

PACIFIC COAST BRANCH.

Thirty-eighth Meeting.

Stanford University, October 27, 1923.

Members present: Alsberg, Barnett, Becking, Evans, Faber, Fleischner, Hall, Hanzlik, Hewlett, Kofoid, Langstroth, Lipman, Lucas, Manwaring, Martin, Oliver, Ophüls, Pierce, Schmidt, Swain, Weymouth.

Thirty-ninth Meeting.

University of California Hospital, San Francisco, December 12, 1923.

Members present: Addis, Alvarez, Beckwith, Clark, Dickson, Foster, Hall, Hanzlik, Hewlett, Langstroth, Lucas, Ophüls, Smith, Sundstroem, Towne, Walker, Weymouth.

Fortieth Meeting.

Stanford Medical School, February 13, 1924.

Members present: Addis, Alsberg, Barnett, Baumberger, Beckwith, Clark, Cowan, Dickson, Faber, Hanzlik, Hewlett, Langstroth, Lipman, Martin, Ophüls, Schmidt and Sundstroem.

Forty-first Meeting.

University of California, Berkeley, April 12, 1924.

Members present: Alsberg, Alvarez, Baumberger, Becking, Beckwith, Dickson, Foster, Hall, Hanzlik, Kofoid, Lucas, Martin, Oliver, Ophüls, Schmidt, Schulz, Sundstroem, Walker, Weymouth.

It was voted that the local section affiliate with the Pacific Coast Division of the American Association for the Advancement of Science.

MINNESOTA BRANCH.

Thirteenth Meeting.

University of Minnesota, Minneapolis, November 15, 1923.

Members present: Eberson, Fahr, Gortner, Henrici, Larson, Lund, Lyon, Newcomb, Palmer, Pettibone, Scammon, Scott, F. H., Willaman.

Fourteenth Meeting.

University of Minnesota, Minneapolis, December 12, 1923.
Members present: Eberson, Fahr, Henrici, Lund, Lyon, McClendon, Palmer, Scammon, Scott, F. H.

Fifteenth Meeting.

University of Minnesota, Minneapolis, January 9, 1924.
Members present: Eberson, Fahr, Gortner, Harris, Henrici, Hirschfelder, Larson, Lund, McClendon, Scott, F. H.

Sixteenth Meeting.

University of Minnesota, Minneapolis, February 13, 1924.
Members present: Brown, Eberson, Fahr, Gortner, Henrici, Larson, Lyon, Lund, McClendon, Palmer, Scott, F. H.

Seventeenth Meeting.

University of Minnesota, Minneapolis, March 5, 1924.
Members present: Fahr, Gortner, Green, R. G., Henrici, Larson, Lund, Lyon, McClendon, Palmer, L. S., Scott, F. H.

Eighteenth Meeting.

University of Minnesota, Minneapolis, May 7, 1924.
Members present: Eberson, Fahr, Gortner, Greene, Henrici, Hirschfelder, Larson, Lund, Lyon, McClendon, Palmer, Scammon, Scott.

WESTERN NEW YORK BRANCH.

Eighth Meeting.

Clifton Springs Sanitarium, Clifton Springs, N. Y., October 13, 1923.
Members present: Anderson, Bloor, Clough, Dooley, Hubbard, Lathrop, Mattill, Mellon, Thomas, Williams, H. U.

Ninth Meeting.

University of Buffalo Medical School, Buffalo, N. Y., November 10, 1923.
Members present: Atwell, Bloor, Hartman, Hubbard, Lathrop, Mattill, Murlin, Williams.

Tenth Meeting.

University of Rochester, Rochester, N. Y., February 16, 1924.
Members present: Bloor, Clough, Hubbard, Knowlton, Mattill, Mellon, Murlin, Simpson, Whipple.

Eleventh Meeting.

N. Y. State Agricultural Station, Geneva, N. Y., April 5, 1924.
Members present: Anderson, Bayne-Jones, Bloor, Clough, Lathrop, Mattill, Murlin, Simpson.

Twelfth Meeting.

Cornell University, Ithaca, N. Y., May 17, 1924.
Members present: Anderson, Bloor, Fish, Goldberg, Hagan, Hartman, Hayden, Hubbard, Knowlton, Mattill, Maynard, Murlin, Simpson.

PEKING (CHINA) BRANCH.

Third Meeting.

Peking Union Medical College, Peking, China, May 16, 1923.

Fourth Meeting.

Peking Union Medical College, Peking, China, October 23, 1923.
Members present: Congdon, Cruickshank, Liu, Meleney, Robertson, O. H., Schmidt, Ten Broeck, Tsen, Wu, Young.

Fifth Meeting.

Peking Union Medical College, Peking, China, December 7, 1923.
Members present: Cruickshank, Liu, Meleney, Robertson, O. H., Schmidt, Ten Broeck, Tsen, Wu, Young.

Sixth Meeting.

Peking Union Medical College, Peking, China, February 11, 1924.
Members present: Congdon, Cruickshank, Councilman, Bauer, Harrop, Kessel, Meleney, Robertson, O. H., Schmidt, Ten Broeck, Tsen, Wu, Young.

Seventh Meeting.

Peking Union Medical College, Peking, China, March 10, 1924.

Members present: Cruickshank, Bauer, Kessel, Liu, Robertson, O. H., Schmidt, Tsen, Wu.

Eighth Meeting.

Peking Union Medical College, Peking, China, April 10, 1924.

Members present: Congdon, Cruickshank, Harrop, Kessel, Liu, Meleney, Schmidt, Wu.

CONSTITUTION AND BY-LAWS.

CONSTITUTION.

[As adopted February 25, 1903, and amended, April 20, 1904, May 24, 1905, February 21, 1906, April 18, 1906, May 22, 1907, March 17, 1915, November 19, 1919, May 18, 1921, and March 15, 1922.]

ARTICLE I. NAME.

The name of this organization shall be the Society for Experimental Biology and Medicine.

ARTICLE II. OBJECT.

The object of this Society shall be the cultivation of the experimental method of investigation in the sciences of biology and medicine.

This object shall be attained (a) by the holding of meetings at which original communications shall be presented; (b) by the publication of Proceedings.

ARTICLE III. MEMBERSHIP.

SECTION 1. *Eligibility.*—Any person who has accomplished a meritorious original investigation in biology or medicine by the experimental method shall be eligible to membership.

SECTION 2. *Forfeiture.*—A. Any member of this society who may consent to the use of his name in any way that would aid in increasing the sale of any patent medicine, proprietary food preparation, or any similar product *for which, in the opinion of the Council, inaccurate or misleading claims are made,* shall forfeit his membership.

B. Any member who conducts an investigation which, in the opinion of the Council, involves unnecessary suffering or inflicts injury upon any person without the understanding consent of that person or that person's guardian, shall forfeit his membership.

C. Any member who, in the opinion of the Council, conducts an investigation that involves unnecessary suffering by animals, shall forfeit his membership.

SECTION 3. *Nomination and Election.*—A. Each candidate for membership must be nominated by three members.

B. After their eligibility has been determined by the council, nominees may be voted for at any meeting succeeding that at which their names were presented.

C. A three-fourths vote of the ballots cast shall elect.

SECTION 4. *Expulsion.*—Any member may be expelled by a three-fourths vote of the total membership.

SECTION 5. *Honorary Membership.*—A. On recommendation by the Council, members may be elected by ballot to honorary membership.

B. Honorary members shall be exempt from all financial obligation to the Society, and shall retain all privileges of membership except the right to vote at executive sessions, and to hold office.

ARTICLE IV. MEETINGS.

SECTION 1. *Time.*—The Society shall hold regular meetings at least once every two months during the academic year.

SECTION 2. *Annual Business.*—The April meeting shall be the annual business meeting.

SECTION 3. *Program.*—The programs of the meetings shall consist of (*A*) brief presentations, in elementary form, of the essential points of experimental investigations, preferably demonstrations of actual experiments; and (*B*) *of papers to be read by title.*

ARTICLE V. OFFICIALS.

SECTION 1. *Officers.*—The elective officers shall be a President, a Vice-President, a Secretary-Treasurer and two Councilors.

They shall be selected from those members whose scientific work is conducted within the limits of Greater New York.

The Chairman of each local branch shall be *ex officio* a Vice-President of the Society.

SECTION 2. *Council.*—The Council shall consist of the President, the Vice-President, the Secretary-Treasurer and two Councilors, one of whom shall be elected annually to serve two years. Ex-Presidents of the Society shall be permanent members of the Council.

SECTION 3. *Nominations.*—A. At each annual meeting there shall be elected a Nominating Committee consisting of seven members from as many different scientific institutions, to serve for the ensuing year. For the term ending with the annual meeting in 1922, this committee shall be appointed by the President. For all ensuing terms, candidates for service on this committee shall be nominated by a primary vote of the whole Society, and elected, by ballot, by the members in attendance at the annual business meetings. The Secretary of the Society shall act as the secretary of this committee.

B. Every member of the Society shall be eligible to membership in the Nominating Committee, but no one shall be eligible to serve more than two terms in succession.

C. It shall be the duty of the Nominating Committee to present to the Secretary of the Society, before the March meeting, at least one nomination for each of the elective offices.

SECTION 4. *Election.*—A. It shall be the duty of the Secretary to forward to all members of the Society, with the preliminary announcements of the April meeting, an official ballot giving a list of all the nominations made regularly by the Nominating Committee, with spaces for the insertion of independent votes for the Officers and for primary nominations for members in the new Nominating Committee. With the presentation of this list, the Secretary shall notify the members of the Society that each may participate in the election of all the officers and in the primary nomination of members of the Nominating Committee.

The Secretary shall present to the tellers, appointed by the President to take charge of the election, all signed ballots forwarded by absent members or received from members in attendance.

B. At each annual business meeting, immediately after the announcement of the returns of the primary nominations for membership in the Nominating Committee, the members present shall elect, from those thus formally put in primary nomination, the members of the new Nominating Committee, *at least four of whom shall be resident members.* The term of office shall be for the ensuing year.

SECTION 5. *Term of Office.*—The term of office shall be one academic year beginning with the October meeting, except as specified for members of the Council elected at large from the Society.

SECTION 6. *Duties.*—A. The duties of the officers shall be such as usually devolve on them individually, and also collectively, as an executive committee.

B. The Council shall promptly investigate and report its findings on the eligibility of candidates for membership and on the desirability of each candidate's election.

ARTICLE VI. FINANCIAL.

SECTION 1. *Dues.—The Council shall have authority to fix the amount of the annual dues.*

SECTION 2. *Privileges of membership begin with payment of dues.* Candidates for membership, if elected, shall not be entitled to any of the privileges of membership before they pay the dues for the fiscal year in which their election occurs.

SECTION 3. *Penalty for non-payment of dues.*—Members in arrears for dues for a period of three consecutive years shall thereupon forfeit their membership, but may be reinstated by the Council.

ARTICLE VII. QUORUM.

Twenty members shall constitute a quorum for the transaction of business.

ARTICLE IX. BY-LAWS.

By-laws may be adopted at any meeting by a majority vote.

ARTICLE VIII. LOCAL BRANCHES.

SECTION 1. *Formation.—Groups of members of the Society shall be allowed by special vote to form local branches, such branches to be designated by local names.*

SECTION 2. *Membership.—Only members of the Society as a whole, duly proposed and elected according to the Constitution, shall become members of the local branches.*

SECTION 3. *Repesentation on the Council.*—Each local branch shall elect a chairman who shall be *ex officio* member of the Council of the Society with the rank of Vice-President.

SECTION 4. *Programs and Publications.—The expense of such local meetings and the preparation of the programs shall be assumed by the local branches. The publication of their proceedings shall take place in the Proceedings of the Society.*

ARTICLE X. AMENDMENTS.

SECTION 1. Proposed amendments of the constitution must be endorsed by at least three members, at a regular meeting, and may be voted on at a succeeding meeting.

SECTION 2. It shall be the duty of the secretary to give all members due notice of intended amendments.

SECTION 3. A two thirds vote of the total membership, or a unanimous vote of the members present, shall be required for the adoption of an amendment.

BY-LAWS.

Adopted February 25, 1903, and amended May 24, 1905, February 21, 1906, March 17, 1915, and May 18, 1921.

I. *Meetings.—A. Regular meetings shall be held on the third Wednesday of the months of October to May inclusive.*

B. Special meetings may be called by the Council.

C. The meetings shall be opened at 8:30 P. M.; two hours shall be devoted to the reading of papers.

D. When possible the meetings shall take place in suitable laboratories.

II. *Editorial Committee.—The President shall appoint each year an editorial committee consisting of the Secretary-Treasurer and two members of the Society. This committee shall have immediate supervision over the preparation of the program and discussion, shall devise rules governing the publication of papers in the Proceedings of the Society, and shall fix the price of the Proceedings to non-members.*

III. *Program.—A. The first eight papers presented to the Secretary to be read in person shall constitute the scientific program for the meeting; all other reports shall be read by title or with the consent of the author shall be transferred to the program of the succeeding meeting.*

B. Precedence on the program shall be given to non-resident members and to demonstrations.

C. No member shall be allowed to read more than one paper at each meeting unless in the event of lack of other material to fill the program.

D. Precedence shall be given, on each program, to communications which have not been presented before any other body and which have to do with investigations essentially experimental in character.

IV. *Regulation of Reports and Discussions.*—A. The time allowed for making individual communications, except demonstrations of experiments, shall be restricted to ten minutes.

B. Not more than five minutes shall be allowed to a member for the discussion of any communication.

V. *Procedure for the determination of guilt under charges affecting tenure of membership.*—A. Any member who may be charged formally and in writing with conduct rendering him subject to the

the constitutional provisions of forfeiture of, or expulsion from, membership, shall be given ample opportunity to show to the Council that the said charges are unfounded.

B. If, after a full hearing, the Council finds, by a majority vote of its total membership, that a member is guilty of conduct rendering him subject to the constitutional provisions relative to forfeiture of, or expulsion from, membership, the Council shall, in the first instance, promptly make due formal announcement of such forfeiture; or, in the second instance, shall formally propose the expulsion of that member, with a statement of the reasons therefor, by a mode of procedure in accord with the general provisions of Art. III, Sec. 4, of the Constitution, but in a manner that will adequately afford the member involved every privilege of appeal to all the members of the Society against the proposed expulsion.

VI. *Order of Procedure, to be followed at the regular meetings:*

A. Call to order.
B. Reading of minutes.
C. Report of Council.
D. Report of committees.
E. Unfinished business.
F. Election of members.
G. Nominations for membership.
H. New business.
I. Scientific program.
J. Adjournement.

REGISTER OF NAMES AND INSTITUTIONAL CONNECTION OF THE MEMBERS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

HONORARY MEMBERS.

Councilman, William T.	Harvard University
Reichert, Edward T.	University of Pennsylvania
Welch, William H.	Johns Hopkins University

ACTIVE MEMBERS.

Abbott, Alexander C.	University of Pennsylvania
Abel, John J.	Johns Hopkins University
Adami, J. George	University of Liverpool, England
Addis, Thomas	Stanford University Medical School, San Francisco
Adler, Herman M.	Juvenile Psychopathic Institute, Chicago
Alexander, Harry L.	Cornell University Medical College, N. Y. City
Allen, Bennet M.	Los Angeles, Calif.
Allen, Edgar	University of Missouri
Alsberg, Carl L.	Leland Stanford University
Alvarez, Walter C.	University of California Medical School
Amberg, Samuel	Mayo Clinic, University of Minnesota
Amoss, Harold L.	Johns Hopkins Hospital
Anderson, John F.	Rutgers College
Anderson, Rudolph J.	N. Y. Agricultural Experiment Station
Atchley, D. W.	Presbyterian Hospital, N. Y. City
Atwell, Wayne J.	University of Buffalo, N. Y.
Auer, John	St. Louis University
Austin, J. Harold	University of Pennsylvania
Avery, O. T.	Rockefeller Institute, N. Y. City

Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Cornell University Medical College, N. Y. City
Bailey, C. H.	Columbia University
Bailey, Cameron V.	N. Y. Post-Graduate Medical School
Bailey, Harold C.	Cornell University Medical College, N. Y. City
Baitsell, George A.	Yale University
Balls, A. K.	University of Pennsylvania
Banta, A. M.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Banzhaf, Edwin J.	N. Y. Health Department
Barber, W. Howard	New York University
Barbour, Henry G.	University of Louisville
Bardeen, Charles R.	University of Wisconsin
Barnett, George D.	Leland Stanford University
Barr, David P.	Washington University School of Medicine

Bauer, J. H.	Peking Union Medical College, China
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumberger, J. Percy	Leland Stanford University
Bayne-Jones, S.	University of Rochester
Bazett, H. C.	University of Pennsylvania
Becking, L. B.	Leland Stanford University
Beckwith, T. D.	University of California
Bell, E. T.	University of Minnesota
Benedict, S. R.	Cornell University Medical College, N. Y. City
Berg, William N.	Berg Biological Laboratory, Brooklyn, N. Y.
Bergeim, Olaf	University of Illinois
Bergey, David H.	University of Pennsylvania
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Binger, Carl A. L.	Hospital of the Rockefeller Institute
Blake, F. G.	Yale University
Blakesley, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blatherwick, Norman R.	Potter Metabolic Clinic, Santa Barbara
Bloor, W. R.	University of Rochester
Boeck, William C.	Harvard University
Boots, Ralph H.	Rockefeller Institute, N. Y. City
Bostrom, Ernest F.	New York University
Brewer, Robert N.	Syracuse University
Bronfenbrenner, J.	Rockefeller Institute, N. Y. City
Brooks, Clyde	University of Alabama
Brooks, Harlow	New York University
Brooks, Matilda M.	Hygienic Laboratory, Washington, D. C.
Brooks, S. C.	Hygienic Laboratory, Washington, D. C.
Brown, E. D.	University of Minnesota
Brown, J. Howard	Johns Hopkins University
Brown, Wade H.	Rockefeller Institute, N. Y. City
Browne, W. W.	College of the City of New York
Bull, C. C.	Johns Hopkins University
Bunting, C. H.	University of Wisconsin
Burnett, Theodore C.	University of California
Burr, Harold S.	Yale University
Burrows, M. T.	Washington University Medical School
Burton-Opitz, Russell	Columbia University
Byrne, Joseph	Fordham University

Calkins, Gary N.	Columbia University
Cannon, Walter B.	Harvard Medical School
Carlson, A. J.	University of Chicago
Carter, Edward P.	Johns Hopkins Hospital
Caulfield, A. H.	University of Toronto, Canada
Cecil, R. L.	Bellevue Hospital, N. Y. City
Chace, Arthur F.	N. Y. Post-Graduate Medical School
Chambers, Robert	Cornell University Medical College, N. Y. City
Chidester, F. E.	University of West Virginia
Child, C. M.	University of Chicago

Chittenden, R. H.	Yale University
Churchman, John W.	Cornell University Medical College, N. Y. City
Clark, Guy W.	University of California
Clark, P. F.	University of Wisconsin
Clough, Harry	University of Rochester
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.	Cornell University Medical College, N. Y. City
Cohen, Barnett	U. S. Hygienic Laboratory, Washington, D. C.
Cohen, Martin	N. Y. Post-Graduate Medical School
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cole, L. J.	University of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Clark University, Worcester, Mass.
Coleman, Warren	New York University
Collett, Mary E.	Ashland, Mass.
Collins, Katharine R.	Spartansburg General Hospital, South Carolina
Collip, J. B.	University of Alberta
Congdon, Edgar D.	Peking Union Medical College, China
Conklin, E. G.	Princeton University
Cooke, J. V.	Washington University Medical School
Coombs, Helen C.	New York University
Cori, Karl F.	State Institute for Study of Malignant Disease, Buffalo, N. Y.
Corner, George V.	University of Rochester
Cowan, George V.	Stanford University Medical School, San Francisco
Cowgill, George R.	Yale University
Crampton, C. Ward	N. Y. Post-Graduate Medical School
Crile, George W.	Western Reserve University
Crocker, William B.	Thompson Institute, Yonkers, N. Y.
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Croll, Hilda M.	Yale University
Crozier, W. J.	Rutgers College
Cruickshank, E. W. H.	Peking Union Medical College, China
Csonka, F. A.	Cornell University Medical College, N. Y. City
Cullen, Glenn E.	University of Pennsylvania
Cunningham, R. S.	Johns Hopkins University
Curtis, Maynie R.	Columbia University
Cushing, Harvey W.	Harvard Medical School

Dakin, H. D.	Ossining, N. Y.
Daniels, Amy L.	Children's Hospital, The State University of Iowa
Danzer, Charles S.	Brooklyn, N. Y.
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davies, H. Whitridge	Rockefeller Institute, N. Y. City
De Bord, George G.	Iowa State College, Ames, Iowa
Detwiler, S. R.	Harvard University
Deuel, Harry J. (Jr.)	Cornell University Medical College, N. Y. City
Dickson, E. C.	Stanford University Medical School
Doan, Charles A.	Johns Hopkins Medical School
Dochez, A. R.	Fresbyterian Hospital, N. Y. City

Doisy, Edward A.	St. Louis University School of Medicine
Dolley, David H.	St. Louis University Medical School
Donaldson, H. H.	Wistar Institute, Philadelphia
Dooley, M. S.	Syracuse University
Dragstedt, Lester R.	Northwestern University Medical School
Draper, George W.	Columbia University
Draper, John W.	New York City
Dresbach, M.	Albany Medical College, Albany, N. Y.
Dubin, Harry E.	Metz Laboratories, N. Y. City
DuBois, E. F.	Cornell University Medical College, N. Y. City
Duggar, B. M.	Missouri Botanical Gardens
Dunn, Halbert L.	Mayo Clinic, Rochester, Minn.
Dunn, Max S.	University of California
Dutcher, R. Adams	Pennsylvania State College
Duval, C. W.	Tulane University

Eberson, Frederick C.	University of Minnesota School of Medicine
Eckles, C. H.	University of Minnesota
Eddy, Walter H.	Columbia University
Edmunds, C. W.	University of Michigan
Edwards, D. J.	Cornell University Medical College, N. Y. City
Eggleston, Cary	Cornell University Medical College, N. Y. City
Eggston, Andrew	Manhattan Eye, Ear and Throat Hospital, N. Y. City
Eisberg, Harry B.	New York University
Eisenbrey, A. B.	Western Reserve University
Elsberg, Charles A.	Mt. Sinai Hospital, N. Y. City
Elser, W. J.	Cornell University Medical College, N. Y. City
Embrey, Hartley C.	Peking Union Medical College, China
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erdmann, Rhoda	Berlin, Germany
Erlanger, Joseph	Washington University Medical School
Evans, Herbert M.	University of California
Ewing, E. M.	Napoleonville, La.
Ewing, James	Cornell University Medical College, N. Y. City
Eyster, J. A. E.	University of Wisconsin

Faber, Harold K.	Stanford University Medical School, San Francisco
Fahr, George	University of Minnesota
Falk, K. George	Roosevelt Hospital, N. Y. City
Falk, I. S.	University of Chicago
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Faust, Ernest C.	Johns Hopkins University
Field, Cyrus W.	New York City
Fine, M. S.	Battle Creek, Mich.
Fischer, Albert	University of Copenhagen
Fischer, Martin H.	General Hospital, Cincinnati
Fish, Pierre A.	Cornell University
Fitch, C. P.	University of Minnesota, St. Paul
Fitzgerald, J. G.	University of Toronto

Fleischner, E. C.	University of California, San Francisco
Fleisher, Moyer S.	St. Louis University
Flexner, Simon	Rockefeller Institute, N. Y. City
Flournoy, Thomas	House of Mercy Hospital, Pittsfield, Mass.
Foster, Goodwin L.	University of California
Foster, Nellis B.	New York Hospital, N. Y. City
Frankel, Florence Hulton	New York University
Friedman, G. A.	Columbia University
Funk, Casimir	State Institute of Hygiene, Poland

Gager, C. Stuart	Brooklyn Botanical Gardens, N. Y. City
Gamble, James L.	Harvard University
Gates, Frederick L.	Rockefeller Institute, N. Y. City
Gay, F. P.	University of California
Gaylord, H. R.	Gratwick Laboratory, Buffalo, N. Y.
Gesell, Robert A.	University of Michigan
Gettler, A. O.	New York University
Geyelin, Henry W.	Presbyterian Hospital, N. Y. City
Gibson, R. B.	The State University of Iowa
Gies, William J.	Columbia University
Githens, T. S.	Mulford Company, Philadelphia, Pa.
Givens, Maurice H.	Northwestern Yeast Co., Chicago
Glaser, Otto	Amherst College
Goetsch, Emil	Long Island College Hospital, N. Y. City
Goldberg, S. A.	Cornell University
Goldfarb, A. J.	College of the City of New York
Goldschmidt, Samuel	University of Pennsylvania
Gortner, R. A.	University of Minnesota, St. Paul
Green, Robert G.	University of Minnesota
Greenwald, Isidor	Roosevelt Hospital, N. Y. City
Gregory, Louise H.	Barnard College, Columbia University
Griffith, Fred R., Jr.	University of Buffalo Medical School
Guenther, A. E.	University of Nebraska
Guthrie, C. C.	University of Pittsburgh
Guy, Ruth A.	Yale University

Hagan, William Arthur	Cornell University, Ithaca
Hale, Worth	Harvard Medical School
Hall, Ivan C.	University of California
Halsey, Robert H.	N. Y. Post-Graduate Medical School
Hammett, F. S.	Wistar Institute, Philadelphia, Pa.
Hanzlik, P. J.	Stanford University Medical School, San Francisco
Harris, Isaac F.	Tuckahoe, N. Y.
Harris, J. Arthur	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Harrison, R. G.	Yale University
Harrop, George A., Jr.	Peking Union Medical College, China
Harrow, Benjamin	Columbia University
Hartman, Carl G.	University of Texas
Hartman, F. A.	University of Buffalo, Buffalo, N. Y.

Hartwell, John A.	Cornell University Medical College, N. Y. City
Harvey, E. Newton	Princeton University
Harvey, Samuel C.	Yale University
Hastings, A. Baird	Rockefeller Institute, N. Y. City
Hatai, Shinkishi	Tohoku Imperial University, Japan
Hatcher, R. A.	Cornell University Medical College, N. Y. City
Hawk, P. B.	Powder Point School, Duxbury, Mass.
Hayden, Charles E.	Cornell University
Haythorn, Samuel R.	University of Pittsburgh
Heidelberger, Michael	Rockefeller Institute, N. Y. City
Helmholz, Henry R.	University of Minnesota
Henderson, Lawrence J.	Harvard Medical School
Hendrix, B. M.	University of Texas
Henrici, Arthur T.	University of Minnesota
Hess, Alfred F.	New York University
Hewlett, A. W.	Stanford University Medical School, San Francisco
Hickernell, L. M.	Syracuse University
Hirschfelder, Arthur	University of Minnesota
Hoffman, George L.	Alleghany County Hospital, Pittsburgh, Pa.
Holm, George E.	Department of Agriculture, Washington, D. C.
Holman, W. L.	University of Toronto
Holmes, S. J.	University of California
Holt, L. Emmett, Jr.	Johns Hopkins University
Hooker, Davenport	University of Pittsburgh
Hooper, Charles W.	Brooklyn, N. Y.
Hopkins, J. Gardner	Columbia University
Hoskins, R. G.	Ohio State University
Höst, H. F.	Kristiania, Norway
Howard, Harvey J.	Peking Union Medical College, China
Howe, Paul E.	Department of Agriculture, Washington, D. C.
Howell, William H.	Johns Hopkins University
Howland, John	Johns Hopkins Hospital
Hubbard, Roger S.	Clifton Springs Sanitarium, N. Y.
Huber, G. Carl	University of Michigan
Hunt, Reid	Harvard University
Hunter, Andrew	University of Toronto
Hurwitz, Samuel	University of California, San Francisco
Jackson, C. M.	University of Minnesota
Jackson, D. E.	University of Cincinnati
Jackson, Holmes C.	New York University
Jacobs, Walter A.	Rockefeller Institute, N. Y. City
Janney, Nelson W.	Los Angeles, Calif.
Jennings, H. S.	Johns Hopkins University
Jobling, J. W.	Columbia University
Jones, Frederick S.	Rockefeller Institute, Princeton, N. J.
Jordan, H. E.	University of Virginia
Joseph, Don R.	St. Louis University Medical School

Kahn, Max	Beth Israel Hospital, N. Y. City
Kahn, Morris H.	Beth Israel Hospital, N. Y. City
Kahn, R. L.	Michigan Department of Health, Lansing
Karsner, H. T.	Lakeside Hospital, Cleveland
Kast, Ludwig	N. Y. Post-Graduate Medical School
Kellogg, V. L.	National Research Council, Washington, D. C.
Kendall, E. C.	University of Minnesota, Rochester
Kessel, John F.	Peking Union Medical College, China
Killian, J. A.	N. Y. Post-Graduate Medical School
Kingsbury, F. B.	Metropolitan Life Insurance Co., N. Y. City
Kinsella, Ralph A.	St. Louis University Medical School
Kirkbride, Mary B.	N. Y. State Dep't of Health, Albany, N. Y.
Kirkham, William B.	Springfield College, Mass.
Kleiner, I. S.	N. Y. Homeopathic Medical School, N. Y. City
Kligler, I. J.	Rockefeller Institute, N. Y. City
Kline, B. S.	Western Reserve University
Klotz, Oskar	University of Toronto
Knowlton, Frank P.	Syracuse University
Knudson, Arthur	Albany Medical College
Kober, Philip A.	Kober Chemical Company, Nepera Park, N. Y.
Kocher, R. A.	San Diego, Cal.
Kofoid, Charles A.	University of California
Kolmer, John A.	University of Pennsylvania
Kopeloff, Nicholas	Psychiatric Institute, N. Y. City
Korns, John H.	Peking Union Medical College, China
Kramer, Benjamin	Johns Hopkins University
Krumbhaar, E. B.	Philadelphia General Hospital, Philadelphia
Krumwiede, Charles	New York University
Kruse,, Theophile K.	University of Pittsburgh
Kugelmass, I. Newton	Yale University
Kuntz, Albert	St. Louis University

Ladd, William S.	Columbia University
Lamar, R. V.	University of Georgia
Lambert, R. A.	Faculdade de Medicina e Cirurgia, São Paulo, Brazil
La Mer, Victor K.	Columbia University
Lamson, Paul D.	Johns Hopkins University
Lancefield, D. E.	Columbia University
Landsteiner, Karl	Rockefeller Institute, N. Y. City
Langstroth, Lovell	University of California, San Francisco
Larson, W. P.	University of Minnesota
Lashley, K. S.	University of Minnesota
Lathrop, Carl O.	University of Buffalo
Laughlin, H. H.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Laurens, Henry	Yale University
Leake, J. P.	Hygienic Laboratory, Washington, D. C.
Lee, Frederick S.	Columbia University
Levene, P. A.	Rockefeller Institute, N. Y. City
Levin, Isaac	New York University

Levine, Michael	Montefiore Hospital, N. Y. City
Levinson, Samuel A.	University of Illinois, Chicago
Levy, Robert L.	Presbyterian Hospital, N. Y. City
Lewis, Howard B.	University of Michigan
Lewis, Paul A.	Rockefeller Institute, Princeton, N. J.
Lewis, Robert C.	University of Colorado, Denver
Lieb, C. C.	Columbia University
Lillie, Frank R.	University of Chicago
Lillie, Ralph S.	Nela Research Laboratory, Cleveland
Lipman, Charles B.	University of California
Little, C. C.	University of Maine
Liu, J. Heng	Peking Union Medical College, China
*Loeb, Jacques	Rockefeller Institute, N. Y. City
Loeb, Leo	Washington University Medical School
Loeb, Robert F.	Presbyterian Hospital, N. Y. City
Loevenhart, A. S.	University of Wisconsin
Lombard, Warren P.	University of Michigan
Longcope, W. T.	Johns Hopkins University
Lucas, William P.	University of California Hospital, San Francisco
Lucke, Baldwin	University of Pennsylvania
Luckhardt, A. B.	University of Chicago
Lund, E. J.	University of Minnesota
Lundsgaard, Christen	Medical Clinic A, Copenhagen, Denmark
Lusk, Graham	Cornell University Medical College, N. Y. City
Lyle, W. G.	Roosevelt Hospital, N. Y. City
Lynch, Clara J.	Rockefeller Institute, N. Y. City
Lyon, E. P.	University of Minnesota

Macallum, A B.	McGill University, Montreal
MacDougal, D. T.	Desert Laboratory, Tucson, Arizona
MacDowell, E. Carlton	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Macht, David I.	Johns Hopkins University
Mackenzie, George M.	Presbyterian Hospital, N. Y. City
Macleod, J. J. R.	University of Toronto
MacNeal, Ward J.	N. Y. Post-Graduate Medical School
MacNider, William DeB.	University of North Carolina
McCann, William S.	University of Rochester
McClendon, J. Francis	University of Minnesota
McCollum, E. V.	Johns Hopkins University
McCrudden, Francis H.	Tufts College Medical School
McEllroy, W. L.	University of Pittsburgh
McLean, Franklin C.	University of Chicago
McMaster, Philip D.	Rockefeller Institute, N. Y. City
McMeans, J. W.	University of Pittsburgh
Magath, T. B.	Mayo Clinic, Rochester, Minn.
Maltaner, Frank	N. Y. State Department of Health, Albany
Mandel, Arthur R.	New York University
Mandel, John A.	New York University
Mann, Frank C.	University of Minnesota

Mann, Hubert	United States Veterans' Hospital, N. Y. City
Manwaring, W. H.	Leland Stanford University
Marine, David	Montefiore Hospital, N. Y. City
Marshall, E. K. Jr.	Johns Hopkins University
Martin, E. G.	Leland Stanford University
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Metz, Charles W.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
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Meyer, A. L.	Johns Hopkins University
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Meyer, K. F.	University of California
Miller, C. Philip	Rockefeller Institute, N. Y. City
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Mitchell, O. W. H.	Syracuse University
Moore, A. R.	Rutgers College
Moore, William	American Cyanide Co., N. Y. City
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Morse, Withrow	Jefferson Medical College, Philadelphia
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Niles, Walter L.	Cornell University Medical College, N. Y. City
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Oertel, Horst	McGill University
Olitsky, Peter K.	Rockefeller Institute, N. Y. City

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Ophüls, William	Stanford University Medical School
Opie, Eugene L.	Henry Phipps Institute, Philadelphia
Oppenheimer, B. S.	Columbia University
Ornstein, George G.	Columbia University
Osborne, Thomas B.	Agricultural Exp. Station, New Haven, Conn.
Osterhout, W. J. V.	Harvard University
Ottenberg, R.	Mt. Sinai Hospital, N. Y. City

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Page, Irvine H.	Cornell University Medical College, N. Y. City
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Palmer, W. W.	Presbyterian Hospital, N. Y .City
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Penfield, Wilder G.	Presbyterian Hospital, N. Y. City
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Thomas, J. E.	St. Louis University
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Thomas, Walter S.	Clifton Springs Sanitarium, N. Y.
Thro, William C.	Cornell University Medical College, N. Y. City
Tisdall, Frederick F.	University of Toronto
Tolstoi, Edward	Russell Sage Institute of Pathology, N. Y. City
Torrey, Harry B.	University of Oregon
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Tsen, Edgar T. H.	Epidemic Prevention Bureau, Peking, China
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Wallace, George B.	New York University
Warthin, Alfred S.	University of Michigan
Wasteneys, H.	University of Toronto
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Weiss, Charles	Research Institute of Cutaneous Medicine, Philadelphia, Pa.
Weiss, Harry	Columbia University
Weiss, Soma	Cornell University Medical College, N. Y. City
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West, Randolph	Presbyterian Hospital, N. Y. City
Weymouth, Frank W.	Leland Stanford University
Whipple, George H.	University of Rochester
White, G. Benjamin	Antitoxin and Vaccine Laboratory, Boston
White, Orlando E.	Brooklyn Botanical Garden, Brooklyn, N. Y.
Wiggers, Carl J.	Western Reserve University
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Williams, Anna W.	Department of Health, N. Y. City
Williams, Horatio B.	Columbia University
Williams, H. U.	University of Buffalo
Williams, J. R.	Highland Hospital, Rochester, N. Y.
Williams, Robert R.	Western Electric Company

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Wilson, Edmund B.	Columbia University
Winslow, C.-E. A.	Yale University
Winternitz, Milton C.	Yale University
Wislocki, George B.	Johns Hopkins University
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Wolf, C. G. L.	Addenbrooke's Hospital, Cambridge, England
Wollstein, Martha	Babies' Hospital, N. Y. City
Wood, Francis C.	Columbia University
Woodruff, L. L.	Yale University
Wu, Hsien	Peking Union Medical College, China

Yatsu, Naohide	Zoological Institute, Tokyo, Japan
Yerkes, Robert M.	Washington, D. C.
Youland, William E., Jr.	N. Y. Homeopathic Medical College
Young, C. C.	Department of Health, Lansing, Mich.
Young, Charles W.	Peking Union Medical College, China

Zingher, Abraham	Department of Health, N. Y. City
Zinsser, Hans	Harvard University
Zucker, Theodore	Columbia University

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*Deceased.

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Secretary	Gies	Gies	Gies	Gies	Gies	Gies

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President	Lee	Morgan	Morgan	Ewing	Ewing	Lusk
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Additional members of Council	Gies Auer	Auer DuBois	DuBois Wallace	Wallace Sherman	Sherman Jobling	Jobling Hess

	1921-'22	1922-'23	1923-'24	1924-'25
President	Wallace	Wallace	Jackson, H. C.	Jackson, H. C.
Vice-President	Jobling	Jobling	Jobling	Jobling
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